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Algal Nutrient Availability of Limitation in Lake Ontario During IFYGL. Part I. Available Phosphorus in Urban Runoff and Lake Ontario Tributary Waters

Texas University at Dallas

Prepared for

Environmental Research Laboratory, Duluth, Minnesota

Oct 76

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*In order to reduce printing costs, appendices have not been included in this report. They may be obtained from Project Officer, Nelson A. Thomas, Large Lakes Research Station, 9311 Groh Road, Grosse Ile, Michigan 48138.

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ALGAL NUTRIENT AVAILABILITY AND LIMITATION IN LAKE ONTARIO DURING IFYGL

Part I. Available Phosphorus in Urban Runoff and Lake Ontario Tributary Waters

by

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FOREWORD

Our nation's freshwaters are vital for all animals and plants, yet our diverse uses of water---for recreation, food, energy, transportation, and industry---physically and chemically alter lakes, rivers, and streams. Such alterations threaten terrestrial organisms, as well as those living in water. The Environmental Research Laboratory in Duluth, Minnesota develops methods, conducts laboratory and field studies, and extrapolates research findings.

--to determine how physical and chemical pollution affects aquatic life

--to assess the effects of ecosystems on pollutants

--to predict effects of pollutants on large lakes through use of models

--to measure bioaccumulation of pollutants in aquatic organisms that are consumed by other animals, including man

This project was conducted as part of the International Field Year for Great Lakes Research and consisted of three separate parts, all directed toward providing information needed to assess the factors limiting algal growth in Lake Ontario and the amounts of nitrogen and phosphorus in tributary drainage which would likely become available in the lake. Part I is concerned with a comprehensive study of the amounts of phosphorus entering Lake Ontario from U.S. tributaries which will likely become available in the lake. Particular attention is given to the particulate and organic forms of phosphorus in the major U.S. tributaries to the lake. Part II is concerned with a study of the amounts of available nitrogen entering Lake Ontario from the U.S. tributaries. Part III is concerned with the factors limiting algal growth in Lake Ontario and in the major U.S. tributaries. This report presents Part I of this study. Parts II and III are published as separate reports by the Environmental Protection Agency under the following titles:

Part II: Nitrogen Available in Lake Ontario Tributary Water Samples and Urban Runoff from Madison, Wisconsin

Part III: Algal Nutrient Limitation in Lake Ontario during IFYGL

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ABSTRACT

Urban runoff, precipitation, and river samples from Madison, Wisconsin and New York State were analyzed for various phosphorus forms and the portion of each phosphorus form available for algal use. Total phosphorus, soluble phosphorus, particulate phosphorus, and soluble reactive forms were measured. In addition, acid extractable, base extractable, and anion exchange resin extractable inorganic phosphorus was determined on the particulate fractions.

Algal assay procedures were used to assess portions of the various phosphorus fractions available for Selenastrum capricornutum growth. Availability of particulate phosphorus in urban runoff from Madison, Wisconsin was highly variable ranging from 8 to 55 percent. Genessee River basin urban runoff had from less than 1 to 24 percent of its particulate phosphorus available. Particulate phosphorus from the Niagra, Genesee, Oswego, and Black Rivers showed only 6 percent or less available to this alga. Autoclaving the samples increased the amount of particulate phosphorus available. Precipitation samples usually showed less than 9 percent of the total phosphorus available to Selenastrum capricornutum. Total phosphorus available for algal growth from New York tributaries was highly variable.

About 39, 24, and 15 percent of particulate phosphorus in urban runoff from Madison could be extracted by acid, base, and anion exchange. Results from urban areas in the Genesee River basin in New York were similar. Resin extractions in long-term aerobic dark incubations produced results similar to short-term tests, indicating that physical and chemical rather than microbial mineralization processes were probably the ket factors regulating the release of inorganic P from the runoff particules to the solution phase.

This report was submitted in fulfillment of Contract No. R-800537-02 under the sponsorship of the Environmental Protection Agency. Work was completed as of June, 1975.

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This report is essentially the same as the Ph.D. thesis of William F. Cowen for the University of Wisconsin.

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16. ABSTRACT

Samples of Madison urban runoff, precipitation from Madison and New York State were analyzed for various forms of phosphorus to estimate the algal-available fraction of each of these P forms. Urban runoff particulate P forms from Madison runoff showed acid extractable inorganic P in the range of 33 to 46% of the particulate P. Ranges for the OH^- and for exchange resin extractable inorganic P were 22 to 27 and 13 to 17 % of particulate P, respectively. Runoff from urban areas in the Genesee R. basin (N.Y.) showed acid, base, and resin extractable inorganic P in the ranges of 30 to 48, 18 to 30 and 11 to 25% of particulate P, respectively, in general agreement with the Madison samples. Inorganic P extracted from particulate P by resin in long-term aerobic dark incubations was similar to that extracted by the resin in short-term tests, indicating that physical and chemical rather than microbial mineralization processes were probably the key factors regulating the release of inorganic P from the runoff particles to the solution phase.

17. KEY WORDS AND DOCUMENT ANALYSIS

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SECTION I

INTRODUCTION

The continuing trend toward urbanization in the United States will pose at least a dual threat to the quality of surface water resources with respect to nutrient inputs. The first threat will come from point sources of plant nutrients, such as sewage treatment plant effluents or industrial wastes. The second threat will come in the form of diffuse sources of nutrients, such as storm water runoff. Both threats will contribute to the fertilization, or eutrophication, of surface waters.

A logical approach to the control of eutrophication will require that sources of nutrients be evaluated in terms of quantity and quality. Since phosphorus has been implicated as a probable limiting nutrient in many waters, the loads of total phosphorus discharged by urban runoff have been measured by several workers. These studies have shown that large amounts of phosphorus are present in urban storm water drainage. A few of these studies have included measurements of "dissolved reactive" or "soluble" phosphorus in an attempt to define the fraction of total phosphorus which might be available for algal growth in the receiving water. However, there is still little information on the availability of phosphorus in the insoluble forms found in urban runoff. The purpose of the studies reported here was to investigate the availability of phosphorus forms in urban runoff and to apply the methods developed for urban systems to the study of phosphorus availability in tributary waters to Lake Ontario as part of the International Field Year for the Great Lakes.

SECTION II

CONCLUSIONS

1. Particulate phosphorus (PP) of 0.45 micron size or larger, isolated from Madison urban runoff, showed group mean values of acid extractable inorganic P from PP (extractable PP_i) which ranged from 33 to 46 percent of PP. The corresponding range for sodium hydroxide extractable PP_i was 22 to 27 percent of PP, and for anion-exchange resin extractable PP_i the range was 13 to 17 percent of PP.

2. Since the group mean values of the chemical extractions represented the chemical nature of PP from the various land uses sampled for urban runoff, the relatively narrow ranges of group mean values for a given type of chemical extraction indicated that the PP forms transported by surface runoff from different land uses in Madison were similar. Possibly, the predominant type of PP in the urban runoff samples was derived from a common source, such as dustfall or eroded soil.

3. Dark incubations of runoff PP in lake waters or dark incubations of unfiltered runoff itself with added anion-exchange resin indicated that physical-chemical processes such as desorption or dissolution were more important factors in the release of inorganic P to solution than was microbial mineralization of PP. This conclusion was the

result of the fairly close agreement between resin extractable PP_i in relatively long-term as compared to short-term aqueous incubation systems containing PP and resin.

4. Bioassays of PP from Madison runoff generally showed availability values which were intermediate between the acid and resin extractable PP_i values for a given sample. An overall bioassay range of 8 to 55 percent of PP was found in 13 samples, 10 of which were derived from runoff draining residentially zoned areas of Madison. The overall average for all samples tested was 30 percent of PP available to Selenastrum capricornutum in 18 days.

5. Because the resin and bioassay tests probably represented the closest approximations to the true availability of PP in the receiving water, their mean values of 15 and 30 percent, respectively, represent a reasonable estimate of the availability of PP in the Madison samples. This range corresponds also to the range of group means reported from base extractions, 22 to 27 percent of PP. Because of particle settling and possibly poor mixing of the runoff particles in the receiving water, these values should be regarded as upper bounds for the availability of PP forms in the receiving water.

6. Soluble P forms in urban runoff, as defined by 0.45 micron filtration, appeared to be subject to overestimation of their algal-available fraction of total soluble phosphorus (TSP) in the Selenastrum bioassays, possibly due to the inherent problems related to the construction of a valid standard curve. However, some of the samples showed less algal-available P than even the chemically measured dissolved reactive phosphorus (DRP) in the runoff filtrates. In these samples, the presence of colloidal, acid-soluble P forms could have inflated the chemical

results from DRP analysis. Care should be exercised in the interpretation of chemical analyses of apparently soluble P forms in runoff, unless the results have been checked with bioassay data.

7. Bioassay of three samples of PP from Madison snow showed PP availability in the range of <2 to 23 percent of PP. The lowest availability value was found in the PP from snow collected in the commercial district of central Madison, where the automobile traffic was very heavy. Consequently, the presence of heavy metal (i.e. lead) toxicity in the algal assay was considered a possible explanation for the lack of growth in this sample.

8. Some of the rain gage samples from the state of New York contained large quantities of phosphorus forms in the <0.45 micrometer size fraction which did not react with the color reagent for DRP nor were used by Selenastrum for growth. In terms of total P availability in the rain waters, only 3 of the 13 samples bioassayed showed 10 percent or more of the TP to be available to Selenastrum. Accurate bioassays of TP were not possible for five of the rain samples because of their low TP levels.

9. Urban runoff samples from two stations in the Genesee River basin in New York showed group mean values of acid, base, and resin extractable PP_i as follows: acid, 30 to 48 percent of PP; base, 18 to 30 percent, and resin, 11 to 25 percent. These values were close to the ranges reported for Madison urban runoff PP samples.

10. The bioassay of PP forms from the Genesee R. basin samples generally showed values which were less than or equal to the resin extraction values, unless the particles were autoclaved before bioassay. Based on resin extraction and bioassay data averages from all samples, probably 16

percent of the PP or less would be expected to become available in the receiving water. The base extraction data predicted a value of about 22 percent of PP.

11. Samples of runoff particles from cropland, pasture, and brushland did not appear to differ significantly from runoff particles from one of the urban stations (Dansville, No. 7), in terms of the PP_i extracted by acid, base, or resin. Particles from the other urban station (Rochester East, No. 2) appeared to show higher percentages of chemically extracted PP_i than did the particles from all other stations.

12. Chemical extractions of Genesee R. particles showed variable fractions of extractable PP_i. However, algal bioassays of these particles indicated that less than 6 percent of the PP was available to *Selenastrum* in 18 days. Autoclaving of the particles resulted in significant increases in the fraction of PP available to *Selenastrum*.

13. None of the PP samples from New York tributaries to Lake Ontario tested by algal growth assays showed more than 6 percent of PP to be available for algal growth on a short-term basis (assay of natural particles). On a long-term basis (assay of autoclaved particles), the assays showed that perhaps 26 to 57 percent of PP may become available in Lake Ontario after death and autolysis of the native organisms associated with the PP in the rivers.

14. Chloroform treatment of unfiltered river waters demonstrated the release of DRP from PP forms in most of the samples tested, indicating the presence of algae and/or zooplankton in the sample PP. The maximum DRP contribution from PP as a result of chloroforming was estimated to represent from 0 to 86 percent of PP in the Niagara R. samples, 3 to 18 percent of PP in the Genesee R. samples, 0 to 60 percent of PP in the Oswego R. samples, and 10 to 20 percent of PP in the Black R. samples. Dark incubations

of unfiltered river water generally showed lower values of DRP contributed by PP than were seen in the chloroform tests, although there were some exceptions to this rule.

15. Because of the possible resorption of DRP to particulate matter after autoclaving, the direct algal assay of autoclaved PP forms in the Algal Assay Procedure - P medium appeared to give a more realistic estimate of the expected PP availability than did the autoclave-filtration bioassay on whole water samples, where the contribution of PP was calculated from the bioassay of the autoclaved sample filtrate and the initial TSP value of the sample.

16. Total P availability showed a wide range of values in the Niagara and Genesee R. samples collected during the study because of changing proportions of PP and TSP in the samples. In the case of the Oswego and Black River samples, however, an estimated TP availability of about 50 to 80 percent (Oswego R.), and 20 to 50 percent (Black R.) was found.

SECTION III

RECOMMENDATIONS

This study has shown that for the Lake Ontario drainage basin and to some extent the urban drainage entering Lake Mendota in Wisconsin a substantial part of the total phosphorus present in urban and rural drainage is not likely to become available for algal growth. It appears that based on this study approximately 20 percent on the average of the non-soluble orthophosphate present in surface water drainage is available for algal growth. These results raise serious questions about the validity of the commonly used approach that the total phosphorus entering a lake or stream from diffuse sources will become available for aquatic plant growth. As a result of these studies, the following are recommended:

1. The best available estimate of aquatic plant available phosphorus entering a given water course be computed by determining the soluble orthophosphate plus 20 percent of the phosphorus that is measured as the difference between soluble orthophosphate and total phosphorus.
2. Because of the impetus currently being given for a national phosphate control program, studies of the type conducted in the Lake Ontario drainage basin and in Madison, Wisconsin whould be expanded to include samples from all of the major rivers in the U.S. before any kind of national phosphate control program is initiated. There is little point in spending large

amounts of money to control unavailable phosphorus.

3. The ion exchange incubation technique and algal assay procedure used in this investigation should be used to evaluate potentially available phosphorus. Other chemical methods such as mild acid or basic extraction procedures overestimate the amount of available phosphorus and therefore should not be used.

SECTION IV

LITERATURE REVIEW

INTRODUCTION TO THE CHEMISTRY OF PHOSPHORUS IN NATURAL WATERS

Phosphorus (P) is present in a multitude of forms in natural waters. However, the majority of these forms contain the phosphorus atom in combination with oxygen as the orthophosphate $(PO_4)^{-3}$ group. This group is also called inorganic P or soluble orthophosphate, and it exists primarily as $H_2PO_4^{-1}$ or HPO_4^{-2} at the pH values commonly encountered in natural waters. Soluble orthophosphate is readily removed from solution by algal cells and can be directly utilized for cell growth. Hence, soluble orthophosphate is "readily available" to algae. All other forms of phosphorus are relatively less available to algae than is soluble orthophosphate, unless the forms can be converted to soluble orthophosphate at a sufficiently rapid rate such that the rate of algal growth is not limited by the rate of conversion. These other relatively "less available" forms include particulate inorganic, condensed, and organic P; and soluble condensed and organic P.

Particulate inorganic P includes the inorganic P found in discrete mineral compounds such as apatite ($Ca_{10}(OH)_2(PO_4)_6$), wavellite ($Al_3(OH)_3(PO_4)_2$), and strengite ($FePO_4.2H_2$)). The group also includes inorganic P associated with amorphous, poorly soluble iron or aluminum oxides and hydroxides which may be in combination with suspended soil particles in the water. The availability of particulate inorganic P forms to algae depends upon sorption-desorption and solution-precipitation processes, in which inorganic P is transferred between the solid and solution phases.

Particulate organic P forms of biological interest generally have inorganic P in combination with carbon as a phosphate ester: $-\overset{|}{\underset{|}{C}}-OPO_3$. The cells of living and dead organisms, as well as organic P esters adsorbed or precipitated on solid particles are included in the particulate organic P class. In order to become available for algal growth, particulate organic P compounds must be hydrolyzed to form organic $-\overset{|}{\underset{|}{C}}OH$ compounds plus soluble inorganic P. Two mechanisms are possible. The organic P compound may be released to solution by desorption, cell lysis, or dissolution reactions, with subsequent hydrolysis of the organic P in the solution phase, or the particulate organic P compound may be hydrolyzed and the inorganic P product released to solution.

It is clear that soluble organic P compounds form a pool of potentially available P for any algal species with the enzymes (phosphatases) necessary for the hydrolysis of the compounds. Soluble condensed phosphates would form a similar pool of potentially available P. Condensed phosphates are composed of inorganic P groups linked by P-O-P bonds, and they can be hydrolyzed by chemical and enzymatic means, producing inorganic P in solution. Condensed phosphates associated with particulate matter would become available for algal growth only after reactions similar to those described above for particulate organic P forms.

Because the chemical characterization of all the P compounds in natural waters is extremely difficult, the P forms have instead been classified in terms of routine laboratory operations. Soluble forms are defined by filtration through a small pore-size filter, generally 0.45 microns in pore size. The soluble forms are then classified according to chemical reactivity to a colorimetric test with acid molybdate. Compounds which react positively are called dissolved reactive P (DRP). This class includes soluble inorganic P or orthophosphate, and possibly colloidal, acid-soluble particulate inorganic P forms or soluble organic P forms which are hydrolyzed in the acidic colorimetric test (Chamberlain and Shapiro, 1973). Generally, however, DRP is felt to be a good estimate of "readily available" phosphorus in a water sample.

Estimation of soluble orthophosphate plus soluble condensed phosphates is generally provided by treatment of a filtered sample with hot acid before colorimetric analysis for soluble inorganic P. The result of such a test is often called soluble hydrolyzable P. Digestion of a filtered sample with perchloric or persulfuric acids releases inorganic P from organic P compounds besides hydrolyzing condensed phosphates. Thus, total soluble P (TSP) is measured with such digestion methods.

Hot acid treatment of an unfiltered sample results in an estimation of total hydrolyzable P, which includes soluble hydrolyzable P forms plus insoluble condensed phosphates and particulate inorganic P dissolved by the acid treatment. The particulate organic P forms can also be measured if the unfiltered sample is treated by a perchloric or persulfuric acid digestion, resulting in an estimation of total P (TP). Subtraction of TSP from TP gives an estimate of the particulate (organic and inorganic) P (PP) in the sample.

SOURCES OF PHOSPHORUS TO URBAN RUNOFF

Precipitation, dustfall, vegetation, street litter, and eroded soil probably account for most of the phosphorus carried from urban areas to lakes and streams by stormwater runoff. Each of these sources will be discussed below, in terms of their importance and of the relative availability of the phosphorus forms which they might contribute.

Precipitation

Very little data has been collected on the concentration of phosphorus in urban precipitation. Weibel *et al*. (1966) measured an average concentration of 80 μgP/l as total hydrolyzable P in precipitation collected in Cincinnati. Kluesener (1971) reported an average of 32 μgP/l, with a range of 8 to 90 μgP/l as total P for rainfall near Lake Wingra in Madison, Wisconsin. In the same study, dissolved reactive P was measured at about 25 μgP/l. Since dissolved reactive P would be expected to be "readily available" for algal growth, about 78 percent of the total P measured in rainfall by Kluesener was apparently "readily available." Tamm (1951) measured a total P level of about 30 μgP/l in rainfall collected just outside of Stockholm, Sweden.

In terms of phosphorus yields, urban rainfall was estimated by Sonzogni and Lee (1972) to contribute 0.20 lbs P/acre/year of total P and 0.16 lbs P/acre/year of dissolved reactive P. Chalupa (1960) noted a much lower yield for a reservoir in Czechoslavakia. He computed that 0.008 lbs P/acre were brought in by rainfall over a seven month period. Allen *et al*. (1968) reported a range of 0.18 to 0.88 lbs P/acre/year for rainfall at stations 10 to 29 km from large towns and industries in Great Britain.

Dustfall

Much of the phosphorus in urban precipitation may be derived from the inclusion of atmospheric dust particles. During dry periods these particles settle out on urban areas, where they await transport by runoff events. A recent study by the American Public Works Association (APWA, 1969) in Chicago recorded an average of 36.9 tons of dustfall/mi^2/month. The dust was 20 to 40 microns in diameter and varied in yield throughout the year; August had the lowest and March the highest yields. Sartor and Boyd (1972) collected composite samples of dry materials from the streets of Milwaukee, Bucyrus (Ohio), and Baltimore. Approximately 56 percent of the total P in the composites was associated with particles less than 43 microns in diameter. They assumed that particles of this size range could have been produced by industrial stacks and vents or raised as dust on construction sites and dispersed by air currents. In Seattle, Johnson et al. (1965) placed jars of water on roofs throughout the city to trap dustfall. The water-soluble P leached from the dustfall ranged from 0 to 0.45 lbs P/acre/year. Kluesener (1971) trapped dustfall to Lake Wingra and calculated yields of about 0.7 lbs P/acre/year for total P and 0.1 lbs P/acre/year for dissolved reactive P. Thus, only about 14 percent of the total P in the dustfall was probably "readily available" phosphorus.

A possible source of dustfall phosphorus in urban atmospheres may be lead halophosphate, which has been detected in automobile exhaust (Anonymous, 1971; reviewed by Kluesener, 1971). However, Bryan (1970) was unable to show a positive correlation between lead and phosphorus in urban runoff from Durham, North Carolina. Thus, the significance of this source of phosphorus is still in question.

Street Litter

After settling out of the atmosphere, dustfall becomes just one of the components of urban street litter. The American Public Works Association (APWA, 1969) research group classified street litter into five groups, namely: rags, paper, dust and dirt, vegetation, and inorganic matter. From June 19 to August 29, the Chicago street litter had the composition shown in Table 1 (a). The dust and dirt was the predominant type of litter. During this period there were only small differences in the total load of litter between high and low population density areas and the city-wide average. In October, however, the litter composition changed considerably, as shown in Table 1 (b). The great increase in the relative amount of vegetation apparently resulted from fallen leaves. During October, the high population density wards showed significantly less litter than the low density wards, as expected from the differences in the amount of forestation between the two types of urban areas.

The seasonal pattern of street litter loading in the Chicago studies showed a minimum base loading in the summer (June - September). Winter residues added extra loading in spring (March - June), and leaves added to the base load in October - November. During most of the year, October - November excepted, the dust and dirt fraction of street litter was the most significant component, ranging from 45 to 83 percent of the total litter by weight. In terms of absolute loads, total litter varied from 0.5 to 8.0 lbs/day/100 ft. of curb. The loads of dust and dirt varied with the type of urban land use, as shown in Table 2. The residential areas showed increasing amounts of dust and dirt as the population density increased, and industrial areas showed the highest loads. Sartor and Boyd (1972) found similar characteristics, for total solids loading as a function of land use, except that commercial areas showed lighter loads than residential areas.

Table 1. COMPOSITION OF CHICAGO STREET LITTER

Litter in Various Materials				
Rags	Paper	Dust and Dirt (% by weight)	Vegetation	Inorganic Matter
(a) June 19 - August 29, 1967				
0.2	4.7	72.0	11.1	12.0
(b) October, 1967				
0.1	2.4	36.5	55.0	6.0

Source: APWA, 1969

Physically, dust and dirt was operationally defined in the Chicago study as that material passing a 1/8 inch mesh cloth. Laboratory leaching tests in a mixing apparatus demonstrated that approximately three percent by weight was soluble in water. The phosphorus content of the leachate averaged 17 μg P/g of solids leached; however, the phosphorus content of the particles was not measured and thus no estimates of the availability of particulate P could be made.

Vegetation

Table 1 (b) demonstrates the importance of vegetation to street litter loads during the fall of the year.

Table 2. DUST AND DIRT LOADS IN CHICAGO

Land Use	Dust and Dirt Loads (lbs/day/100 ft. of Curb)
Single Family Residential	0.7
Multiple Family Residential	2.3
Commercial	3.3
Industrial	7.8

Source: APWA, 1969

The contribution of phosphorus from detached vegetation (litter) will be discussed below. It is known, however, that living vegetation also has the capacity to contribute phosphorus to runoff, through contact with precipitation. Tamm (1951) noted an increase in the phosphorus concentration of rainfall as a result of passage through a canopy of pine needles or birch leaves. The rain which passed through the canopy, called throughfall, was enriched in phosphorus by three to ten times over the level of phosphorus in the incident rainfall. These tests were made in an area just outside Stockholm, Sweden. In tests conducted in an experimental forest, the enrichment of phosphorus in throughfall was only about two times the control level, for pine and spruce canopies. Tamm concluded that the higher values near Stockholm may have been due to urban dustfall accumulations on the leaves of the trees. Carlisle *et al.* (1966b) analyzed rain and throughfall for an oak canopy. They reported a phosphorus yield for throughfall of 1.1 lbs P/acre/year compared to 0.38 lbs P/acre/year for rainfall controls. Throughfall yields were higher in the period of vegetative growth than in the leafless period, and in May they detected very high yields of phosphorus in throughfall, apparently due to pollen and new leaves. The new leaves were shown to have a higher phosphorus content (0.7 percent) than leaves from the rest of the year (0.2 percent). Will (1959) studied conifer forests in New Zealand and computed throughfall yields of 0.42 to 3.9 lbs P/acre/year compared to rainfall yields of 0.21 to 0.54 lbs P/acre/year.

Upon detachment of leaves or seeds from trees, the phosphorus contained in the vegetative litter becomes a potential source of phosphorus to urban runoff. Inorganic phosphorus occurs in plants primarily in the vacuolar sap as soluble orthophosphate (HPO_4^{-2} and $H_2PO_4^{-1}$), and concentrations in the sap may be hundreds of times greater than the concentration that exists in the soil solution (Lawton, 1961). Organic phosphorus occurs as nucleotides, phosphate-sugar esters, phospholipids, nucleoproteins, and nucleic acids. Seeds are known to contain large amounts of organic phosphorus, particularly inositol hexaphosphate, or phytic acid. In vegetative tissues, however, inorganic phosphorus is usually predominant.

Indirect evidence for the phosphorus contribution of vegetative litter to urban runoff has been supplied by Kluesener (1971), who noted high concentrations of phosphorus in runoff samples collected in May and November. The high spring concentrations were attributed to the leaching of tree seeds by runoff. Although lawn and garden fertilizers were being applied at this time of the year, their contribution was discounted by Kluesener because they were being applied to pervious surfaces where the yield of runoff was insignificant. Carlisle et al. (1966a) pointed out that although small, non-leafy materials such as insect frass, bird scales, and male flowers that fall in the spring accounted for only 14.7 percent of the dry weight of the total annual litter-fall in an oak stand, these materials contributed 40.2 percent of the phosphorus in all types of vegetative litter.

The November peak in runoff phosphorus seen by Kluesener (1971) was attributed to the rainfall or runoff leaching of piles of leaves in the street gutters. Cowen and Lee (1973) leached oak and poplar leaves with distilled water in laboratory columns. Dissolved reactive phosphorus was readily leached from the leaves; when oak leaves were cut to expose veins to the water, the yield of dissolved reactive phosphorus was increased from about 240 µg P/g for whole leaves to 650 µg P/g for the cut leaves (oven-dry weights). Increases in the time of soaking prior to the column leaching were also shown to increase phosphorus yields. Surface films of moisture on leaves collected after a rainstorm were found to contain dissolved reactive phosphorus, which could be carried off the leaves in subsequent runoff events.

Other plant leaching studies by Timmons et al. (1970) have demonstrated that the leaching of soluble phosphorus from plants is greatly enhanced by drying or freezing treatments. Since leaf litter is often subjected to freezing over the winter months in many urban areas, these results indicate that high concentrations of dissolved phosphorus should be found in spring melt waters. The leaching studies indicate that careless disposal of grass clippings or leaf litter by urban residents can greatly enhance the yield of available phosphorus from these materials. Street gutters or storm sewer catchment basins are clearly the worst possible disposal sites, as they enhance the leaching of phosphorus into runoff. Sartor and Boyd (1972) reported concentrations of 1,100 to 2,200 μg P/l (total P) in supernatant water from Milwaukee and Baltimore catch basins.

Eroded Soil

Exposed land in the vicinity of impervious surfaces such as streets or sidewalks has been shown to yield large amounts of sediment to runoff. "Urbanization" by definition means the construction of new homes, roads, and other structures on previously unused or rural lands. Such activities expose soil to the erosive forces of wind and rainfall. Wolman and Schick (1967) compared the sediment loads in streams draining natural or farm lands with loads in streams draining urban areas near Washington, D.C. and Baltimore. The sediment concentrations in rural streams were 2,000 mg/l or less, while urban streams from construction areas varied from 3,000 to over 150,000 mg/l.

Thompson (1970) evaluated the rates of erosion for parts of Detroit and found a rate of 69 tons/acre/year for areas under development, compared with an average rate for the city of only about 3 tons/acre/year.

Storm sewer loads of sediment have been investigated by Benzie and Courchaine (1966), in a comparison of separate and combined sewer system discharges in Michigan. The concentrations of suspended solids in the separate system were 10 times those in the combined system, and the solids were primarily clay resulting from construction and development activities in the drainage basin. Evans et al. (1968) tested the change in the total-P of runoff samples (23 to 2700 μg P/l) during various periods of settling in the laboratory and found that total P was reduced by 30 percent in five hours' time, while suspended solids were reduced by 70 percent during the same time period. This agrees with the findings of Sartor and Boyd (1972), that about 44 percent of the total-P by weight in dry street contaminants was associated with particles over 43 microns in size (referred to as "sand" on the basis of size), and these particles made up 74 to 92 percent of the dry matter by weight in the composite samples from Milwaukee, Bucyrus, and Baltimore. The other 56 percent of the total-P was associated with lighter (less than 43 micron) particles, which have been discussed above in terms of dustfall, and which would not settle as readily as "sand" size material.

The availability of phosphorus associated with eroded soils is a complicated problem, involving both (a) physical-chemical and (b) biological reactions.

Physical-chemical reactions of soils--

Physical-chemical reactions of importance in soils are adsorption-desorption and precipitation-dissolution. Adsorption and precipitation reactions between soil constituents and soluble inorganic or organic phosphates result in phosphate fixation by the soil and a corresponding decrease in the concentration of soluble P forms in the water.

The form of phosphorus in the soil will depend upon the pH, microbial activity, and oxidation-reduction potential of the soil. In acid soil, inorganic P is usually combined with iron and aluminum; Williams *et al*. (1971a) concluded that, in the non-calcareous lake sediments which they studied, inorganic P was probably held by a short range order iron complex. Similarly, Williams *et al*. (1958) indicated the importance of amorphous iron and aluminum in P fixation by soils. In neutral or alkaline soils, inorganic P may be held by fertilizer-soil reaction products or as an apatite mineral. Sorption or precipitation of inorganic P by calcium carbonate has been suggested as another mechanism (Williams *et al*., 1971b).

Because clays may contain up to 50 percent of the total-P in some soils, and because clays are readily eroded (Scarseth and Chandler, 1938) fixation of phosphorus by this soil fraction should be quite important in urban oxide and hydrous oxide coatings on clay mineral surfaces. Consequently, the mechanisms of inorganic P fixation on clays may be similar to those in acid soils. The combination of high P content and susceptibility to erosion results in a high concentration of phosphorus in eroded soil relative to the undisturbed soil. Massey and Jackson (1952) reported concentrations of ph 3-extractable inorganic P in eroded soils which were 3.4 times the concentration of pH 3-extractable inorganic P in the undisturbed soils, in samples from four locations in Wisconsin.

A similar enrichment would be expected for the organic P forms in eroded soils, as up to 85 percent of the total-P in soils may be in organic forms (Alexander, 1961), and concentrations of organic matter in eroded soils have been reported to be 2.1 times the concentrations found in undisturbed soils (Massey and Jackson, 1952).

The principal organic phosphorus compounds in eroded soils are those derived from the tissues of plants and micro-organisms; nucleic acids, phospholipids, and inositol phosphates. The fixation of nucleic acids by clay minerals has been studied by Goring and Bartholomew (1952), who concluded that these compounds were adsorbed by a reversible, cation dependent reaction. The nucleic acids have been estimated to be present in soils at levels of about five to ten percent of the total organic phosphorus (Anderson, 1967). Phospholipids are also a minor fraction of the organic phosphorus, probably about 0.5 to 2.5 percent of the total (Alexander, 1961).

The major identified organic phosphorus forms in soils are the inositol phosphates. Inositol is a six-carbon cyclitol with several isomers, all of which can form phosphate esters containing one to six phosphate groups. The hexaphosphates form iron and aluminum salts which are insoluble in acid media; consequently, the fixation of these compounds is probably analogous to that of inorganic phosphate. Jackman and Black (1951a) found that in the presence of an excess of the respective cations, iron phytate (myoinositol hexaphosphate) was insoluble from pH 2.5 to 8.0 and aluminum phytate was insoluble from pH 3.0 to 9.0. Calcium phytate, in contrast, was insoluble above pH 6.0 and magnesium phytate was insoluble above pH 9.7. The salts of the lower inositol phosphate esters were more soluble than those of the phytates, but still less soluble than salts of orthophosphate. Anderson and Aldridge (1962) showed strong sorption of phytic acid on boehmite, soil clay, and montmorillonite at pH 3.0 to 4.0. Similarly, Goring and Bartholomew (1950) noted that the maximum sorption of inositol hexaphosphate on bentonite, kaolinite, and illite occurred at pH 3.5 to 4.5, which agreed with the minimum of the solubility versus pH curves for the iron and aluminum salts of the inositol hexaphosphate. The same behavior was noted with orthophosphate.

The reverse of P-fixation reactions in soils are desorption and dissolution, which result in an increase of soluble P for possible algal uptake. The balance between P-fixation and P release reactions is partly related to the soluble P concentration existing in the vicinity of the soil particle. Eroded soils are expected to act as phosphate "buffers" by removing or adding P to solution in response to changes in P concentration. Such behavior has been studied by Taylor and Kunishi (1971), Kunishi et al. (1972), and Ryden et al. (1972) for eroded agricultural and urban soils. These authors equilibrated soils in laboratory systems containing various initial concentrations of soluble inorganic P. They then calculated the amount of P (in ppm of dry soil) adsorbed or desorbed by the soil and plotted these values against the final concentrations of soluble inorganic P in solution, after equilibration. The intersection of the curve with the concentration axis gave the equilibrium phosphate concentration, which was the concentration in the water at which no net P uptake or release would take place.

Even more important than the equilibrium phosphate potential, however, was the slope of the curve. The slope was an index of the capacity of the soil to buffer the phosphorus concentration in a solution. Taylor and Kunishi (1971) defined the biologically available P in the soil as the amount of P which must be removed from the soil in bringing the soil into equilibrium with an arbitrarily chosen concentration of P in the water phase. If the chosen concentration of soluble inorganic P were very low, as in the epilimnion of a productive lake in late summer, the biologically available P measured in the test might well be close to the entire amount of potentially available P in the soil.

The kinetics of such buffering action have been investigated by Li et al. (1972), using lake sediments equilibrated with soluble inorganic ^{32}P. They reported that a major portion (45 to 87 percent) of the exchangeable native sediment P participated in a rapid exchange reaction characterized by a first-order (with respect to P) rate constant ranging from 7.4 to 46 hours $^{-1}$. The exchange process was graphically resolved into three first-order reactions of differing rates. Similarly, Amer et al. (1955) have used 32^{P} and anion-exchange resin equilibrations of soils to demonstrate the existence of at least three reactions occurring simultaneously but with different rates of P exchange. The fastest reaction was completed in about 15 minutes, the intermediate reaction in two to three hours, and the slowest reaction was still continuing at the end of 72 hours. Their resin-adsorption data for four soils showed that between 51 and 75 percent of the P eventually adsorbed by the anion-exchange resin from the soils in 72 hours was picked up by the resin in the first two hours of the tests. Such data demonstrates the time dependence of soil availability terminology such as "readily available" and "potentially available."

Biological reactions of soils--

The studies of phosphate exchange cited above did not distinguish the role of microorganisms in the reactions from the physical-chemical factors, although the latter were clearly considered to be the dominant factors influencing the results of P exchange experiments. However, microorganisms have been shown to remove inorganic P from solution in competition with lake muds (Phillips, 1964), and the availability of both inorganic and organic P forms in soils may be determined, on a long-term basis, by microbial solubilization reactions.

For example, many types of microorganisms in soils are capable of solubilizing inorganic phosphorus compounds which are insoluble under normal soil conditions. Alexander (1961) estimated that 10 to 50 percent of the bacterial isolates which he tested could solubilize calcium phosphates. The bacteria apparently produced organic acids, or in the case of chemoautotrophs, nitric or sulfuric acids which dissolved the calcium phosphates. Rodina (1963) stated that bacteria capable of liberating phosphorus from organic compounds and those able to transform insoluble compounds into soluble ones were always present in the detritus of the lakes tested. Recent work by Harrison et al.(1972) on Klamath Lake sediments showed similar results. The order of microbial solubilization was $CaHPO_4 > Ca_3(PO_4)_2 > FePO_4 > Mg(PO_4)_2 > Al\ PO_4$. A chelation mechanism was proposed, in which organic acids produced by the bacteria complexed the cations to release the phosphate to the water.

Microbial reactions of importance to the availability of organic P compounds are the hydrolysis reactions catalyzed by phosphatase enzymes. These reactions split off inorganic P from its esters. If the ester was insoluble, the inorganic P may not be released to solution but instead may be bound by the same mechanisms which held the ester. In this case the availability of the organic P compound would show no change. In the case of dissolved esterified P, however, the action of a phosphatase enzyme would produce soluble inorganic P for immediate uptake by an algal cell.

The investigation of soil-bound organic P hydrolysis has demonstrated the resistance of these forms of enzymatic attack. This is especially true for the phytins, which are hydrolyzed more slowly than phospholipids and nucleic acids (Alexander, 1961). Jackman (1949) felt that it was not the level of phytase enzyme activity, but rather the low solubility of phytin that limited its decomposition in his tests. Likewise, Greaves et al. (1963) estimated that 50 percent of the microorganisms present in soil and on plant roots possess an active phytase enzyme. Bower (1949) discovered a decrease in enzyme activity when kaolin or bentonite was added to a mixture of nuclease enzyme and various nucleic acid derivatives. The extent of decreased activity could not be explained solely by the adsorption of the enzyme by the clays, so that nucleic acid derivatives must also have been sorbed, accounting for the rest of the inactivation.

Two other factors besides solubility which can affect the enzymatic hydrolysis of organic phosphorus are temperature and pH. Bower (1949) found higher rates of hydrolysis at 35°C than at 25°C in soils. According to Thompson and Black (1949), the release of inorganic P responded slightly to increases in temperature below 30°C, while above this temperature the rates were markedly influenced by rising temperature.

The effect of pH on the hydrolysis or organic P in soils is complicated by its effects on enzyme kinetics and on organic P solubility. Jackman and Black (1951b) investigated these effects with inositol phosphates and phytase enzymes at different pH values in soils. Iron and aluminum salts showed very little hydrolysis in the pH range of 3.0 to 7.0 where these salts are slightly soluble. In the case of calcium and magnesium salts, the enzyme activity and hydrolysis rates both showed a maximum at pH 5.0 to 6.0, decreasing rapidly on either side of this pH range. The solubility of the calcium and magnesium salts was low above this pH range, and the salts were completely soluble below this pH range. The hydrolysis rate was apparently maximum at the pH values where both solubility and enzyme activity could be achieved. Halstead (1964) used disodium phenylphosphate as a substrate to assay the phosphatase activity of soil suspensions in buffered solutions. An acid mineral soil showed activity which gradually increased from pH 2.0 to 7.0. An organic soil gave peaks of activity between pH 5.0 and 9.5, indicating the presence of both acid and alkaline phosphatases.

The eventual fate of hydrolyzed inorganic P released from organic P forms in runoff will depend upon the composition of the organic residues undergoing microbial decomposition. Should the concentration of phosphorus in these residues exceed the relative concentration required for heterotrophic bacterial nutrition, the excess phosphate will appear as inorganic P in solution (net mineralization). This phosphorus would be "readily available" to algae in the water receiving the runoff. Alexander (1961) calculated that at least 0.2 percent of the dry weight of decomposing carbonaceous matter should be phosphorus, if net mineralization is to occur. At lower concentrations, net immobilization will occur, as the phosphorus becomes incorporated into microbial tissue. Some of this phosphorus will be released later as soluble inorganic P after death and lysis of the microorganisms.

PHOSPHORUS CONCENTRATIONS IN URBAN RUNOFF

In early studies on urban runoff, the characteristic high levels of suspended solids, biochemical oxygen demand, and coliform counts were sufficient evidence to alert water pollution control workers to the problems of surface runoff. Later workers began to include nutrient analyses in their research, as the concern for eutrophication became stronger.

Sylvester and Anderson (1964) summarized their data from street gutter samples as follows: soluble P (soluble reactive P) had a median value of 22 μgP/l, and total P had a median value of 155 μgP/l. Since "soluble reactive" probably measures readily available phosphorus, about 14 percent of the total P in their samples was in a readily available form.

Weibel _et_ _al_. (1966) reported the range of P concentrations in their Cincinnati study to be 7 to 2,430 μgP/l as total hydrolyzable phosphorus, with a mean value of 370 μgP/l. About 62 percent of the total hydrolyzable P was soluble hydrolyzable P.

Bryan (1970) selected a 1.67 square mile section of Durham, North Carolina, for sampling urban runoff. Phosphorus concentrations were 27 to 157 μgP/l soluble P (mean 50 μgP/l) and 177 to 806 μgP/l total P (mean 400). The ratio of soluble P to total P mean values was 0.13 or 13 percent. Although Bryan did not clearly indicate whether soluble P was measured as reactive P or hydrolyzable P, the computed ratio does give an indication of the available proportion of total P.

Measurements made in Tulsa, Oklahoma (Avco Economic Systems Corporation 1970) indicated a range of 180 to 1160 μgP/l for dissolved reactive P, with a mean of 380 μgP/l. Total P was not measured. Burm _et_ _al_. (1968) sampled storm sewers in Ann Arbor, Michigan, and reported mean annual values of 270 μgP/l for soluble P and 1670 μgP/l for total P. They did not describe the procedure used to measure soluble P.

Kluesener (1971) investigated urban runoff P concentrations in Madison, Wisconsin, and obtained an annual average of 570 μgP/l as dissolved reactive P and 980 μgP/l as total P; indicating that about 58 percent of the total P in his runoff samples was apparently in readily available forms.

If all the values reported in runoff studies as dissolved reactive, soluble, and soluble hydrolyzable are considered to be estimates of P forms which are eventually convertible to soluble orthophosphate in a receiving water, then these values are estimates of "soluble available P." Table 3 compares the runoff studies on the basis of the percentage of total P reported as "soluble available P." About 33 percent was obtained as an overall average from all the studies listed, indicating that perhaps 67 percent of total P is in particulate forms of comparatively unknown algal availability. The concentrations of soluble P forms listed in the table indicate that, without even considering particulate P forms, urban runoff probably contains available P at levels far above the concentration of 10 μgP/l in lake waters cited by Sawyer (1947) as being an upper limit before nuisance growths of algae will occur. Hence, urban runoff would be exerting a phosphorus fertilizing effect on most lake waters, although the magnitude of the effect would depend upon the relative volumes of the lake and of the runoff.

EFFECT OF URBAN LAND USE ON RUNOFF PHOSPHORUS

Based on the small number of areas investigated, phosphorus yields for urban runoff appear to range from 0.21 to 1.5 lbs P/acre/year for total P and 0.4 to 2.7 lbs P/acre/year for soluble P, as listed in Table 4 . The differences among the areas investigated cannot be explained completely based on the area type. Bryan (1970) was not able to relate land used to any of the various pollution parameters in his studies. Moreover, the studies of Kluesener (1971) and Weibel *et al*. (1964, 1966) on residential and residential-light commercial areas, respectively, showed P yields which were in close agreement with the yield from an area containing 20 percent commercial and industrial land uses besides residential, public, institutional, and unused lands (Bryan, 1970). Hence, the relationship between phosphorus yield and urban land use is still not clear.

The distribution of phosphorus sources, in contrast, does seem to be related to land use. The Chicago study by the American Public Works Association (1969) ranked the various urban land used in terms of their dust and dirt accumulations in the following order: industrial > commercial > multiple family residential > single family residential. Sartor and Boyd (1972) found the following loading intensities for total P in the form of dry street surface contaminants (lbs/curb mile): industrial, 3.43; residential, 1.07; and commercial, 0.29. These values reflected the variations in total solids loading intensities, rather than the phosphorus content of the solids, which did not seem to be significantly different in the various land uses.

Table 3. ESTIMATED PERCENT OF TOTAL P AS "SOLUBLE AVAILABLE P" IN URBAN RUNOFF

	(A) "Soluble Avail. P[a]" (μgP/l)	(B) Total P[a] (μgP/l)	(A)/(B) (percent)
Burm *et al.* (1968)	270	1,670	16
Kluesener (1971)	570	980	58
Sylvester & Anderson (1964)	22	155	14
Bryan (1970)	50	400	13
Weibel *et al.* (1966)	230	370	62
		Average	33

[a] Mean values except for Sylvester & Anderson (1964), where median values were reported.

Table 4. PHOSPHORUS YIELDS IN URBAN STORMWATER RUNOFF

Author	P Yield as: Soluble P (lbs. P/acre/yr)	P Yield as: Total P (lbs. P/acre/yr)
Weibel (1969)	--	0.94
Kluesener (1971)	0.6	1.0
Jaworski & Hetling (1970)	--	0.21
Bryan (1970)	--	1.1
Avco (1970)	0.4 to 2.7	--
Owen & Johnson (1966)	--	1.5

PHOSPHORUS IN LAKE ONTARIO TRIBUTARIES

Niagara River

Table 5 shows the five largest waste sources to Lake Ontario, as compiled in a 1969 report to the International Joint Commission (IJC) investigating the lower Great Lakes. The most significant source of phosphorus to Lake Ontario is the Niagara River, with an estimated total P input of 7,700 to 8,000 tons per year, primarily from Lake Erie and from municipal wastes entering the river from Canada and the U.S. (Table 6).

The mean flow of the Niagara River is 195,000 cfs (cubic feet per second), or 85 percent of the flow to the lake from all tributaries. Figure 1 shows the locations of the major urban areas on the river, which are responsible for increasing the concentration of phosphorus in the river by 71 percent between Lake Erie and Lake Ontario (IJC, 1969).

Genesee River

The Genesee River originates in the hills of northern Pennsylvania and flows north across New York to its mouth on Lake Ontario at Rochester, New York. About three-quarters of the 2,446 square mile drainage basin is used for agriculture. The combination of phosphorus from land drainage in the basin and industrial and municipal wastes from the Rochester metropolitan area (into the river or directly into the lake) results in a phosphorus contribution to the lake of about nine percent of the total P input (Table 5).

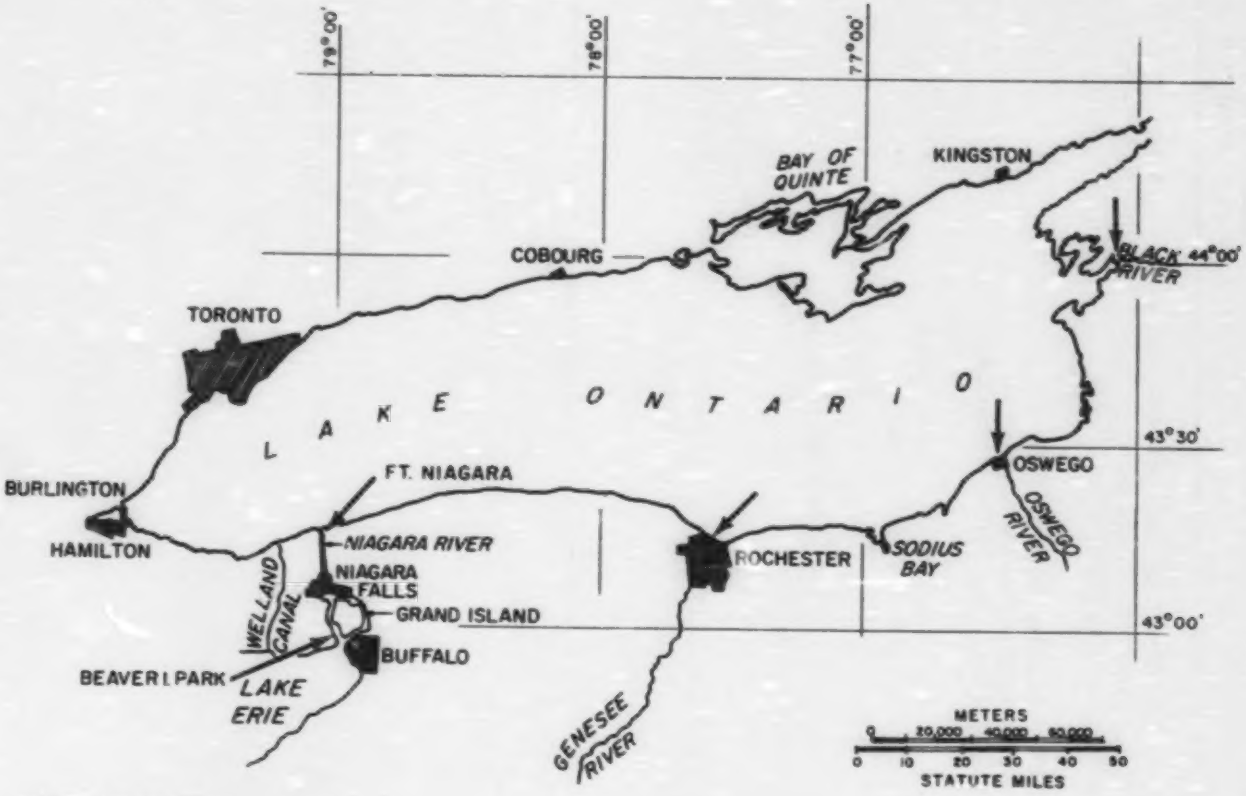
Oswego River

The Oswego River drains about 5,000 square miles of north central New York, including most of the Finger Lakes area. The land uses in the basin are similar to those of the Genesee basin: cropland with pasture, woodland, and

Table 5. MAJOR SOURCES OF PHOSPHORUS TO LAKE ONTARIO (1966-1967)

Source	% of Total P from all sources
Niagara River, including municipal and industrial sources from the Buffalo-Niagara Falls area.	56
Metro Toronto region, including all local municipal, industrial, and tributary discharges to Lake Ontario.	13
Metropolitan Rochester area, including all municipal and industrial waste sources and the Genesee River.	9
St. Catherine area, including municipal and industrial waste sources and Twelve Mile Creek.	5.2
Hamilton area, including municipal, industrial, and tributary discharges to Hamilton Harbour.	2.3

(Source: IJC, 1969)


79°00'
78°00'
77°00'
BAY OF QUINTE
KINGSTON
COBOURG
BLACK RIVER
44°00'
TORONTO
LAKE ONTARIO
43°30'
OSWEGO
OSWEGO RIVER
BURLINGTON
FT. NIAGARA
HAMILTON
NIAGARA RIVER
NIAGARA FALLS
GRAND ISLAND
ROCHESTER
SODIUS BAY
43°00'
WELLAND CANAL
BEAVER I. PARK
BUFFALO
LAKE ERIE
GENESEE RIVER
METERS
0
20,000
40,000
60,000
0
10
20
30
40
50
STATUTE MILES

SOURCE: NOAA (1971)

Figure I. Lake Ontario and major tributaries

Table 6. SOURCES OF PHOSPHORUS TO THE NIAGARA RIVER

Source		Total P (short tons/year)
Lake Erie		4,500[a]
New York State	Municipal Wastes	2,000
	Industrial Wastes	150
	Land Drainage	50
Province of Ontario	Municipal Wastes	330
	Industrial Wastes	80
	Land Drainage	50
Unaccountable		540
	Total	7,700[a]

(Source: IJC, 1969)

[a] A value of 4,800 short tons per year has been estimated for Lake Erie input in 1972 (Great Lakes Water Quality, 1972); this new value would change the total in the table to 8,000 tons per year.

forest. The 1969 IJC report estimated a stream loading from municipal treatment plants and untreated sources in the basin equivalent to a population of 475,000 persons.

Black River

The Black River drainage basin covers an area of about 1,930 square miles on the western slope of the Adirondack Plateau. The upper reaches of the basin are mountainous and wooded, while the region near Lake Ontario is similar to the agricultural lands of the Genesee or Oswego basins.

In terms of phosphorus loads to Lake Ontario, the 1969 IJC report listed the New York rivers in the following order of increasing loads: Black R., 181 tons P/yr.; Genesee R., 314 tons P/yr.; Oswego R., 619 tons P/yr.; and Niagara R., 7,700 tons P/yr. The annual total P input from all sources (tributaries plus direct discharges to the lake) with planned P reduction programs, has been estimated at 17,800 tons in 1972 (Great Lakes Water Quality, 1972), compared to an estimated 13,700 tons in 1969 (IJC, 1969). Since the lake is presently in a stage of nutrient content intermediate between oligotrophy (nutrient-poor) and mesotrophy (intermediate nutrient enrichment), future increases in the phosphorus and nitrogen loadings from tributaries and direct waste discharges could cause major changes in algal production in the lake. Already, the inshore waters near Toronto and the mouths of the Niagara, Genesee, and Oswego Rivers show serious growths of the green alga *Cladophora* and the presence of benthic (bottom-dwelling) organisms which are normally characteristic of eutrophic (nutrient-rich) waters (IJC, 1969).

Phosphorus Loads and Stream Discharge

The rate of both sediment and phosphorus loading from a river basin is not constant throughout the year, but instead correlates with the stream hydrograph. During periods of higher than normal flow, the base flow of the stream is supplemented with surface runoff waters, which generally carry much higher concentrations of suspended sediment and total P than does the base flow. Since both the flow and the average P concentration are increased, the yield per unit time (flow times concentration) must also increase. Owen and Johnson (1966) found highly significant correlations between daily yield of total P and streamflow from six watersheds (urban and agricultural) in the metropolitan Toronto region of the Lake Ontario drainage basin. The association between high total P concentrations and stream discharge from an agricultural watershed was noted by Shannon and Lee (1966) in their studies on Black Earth Creek, Wisconsin. They reported peak concentrations of about 900 μgP/l of total P and 500 μgP/l of soluble orthophosphate plus soluble condensed phosphates, indicating that perhaps 55 percent of the total P was in particulate forms during the period of snowmelt in the watershed.

In addition to inputs of phosphorus from runoff during high flow periods, stream loads are also increased by high rates of streambank erosion and resuspension of streambed sediments up into the wash load carried by the water above the bottom of the stream. Since phosphorus is associated with the streambank and wash load sediments, the increased sediment loads during high flow periods should result in increased particulate phosphorus loads during these periods. In terms of percent of total annual loading of particulate phosphorus to Lake Ontario, the particulate matter carried into the lake during the high spring flow period should be the most important input, and availability studies on this material would probably be more significant than studies on material which enters the lake in smaller quantity during the rest of the year.

ANALYSES OF AVAILABLE PHOSPHORUS

Chemical Methods

Since soluble orthophosphate, or soluble inorganic P, is readily available for algal growth in natural waters, the question of availability as applied to a given phosphorus form can be reduced to the question of how rapidly and to what extent that phosphorus form can supply soluble orthophosphate to an algal cell.

Consequently, attempts have been made to accurately measure soluble orthophosphate, as the best means of estimating the true value of "readily available P" in a water sample. One of the most widely used procedures is the molybdenum blue procedure with an antimony catalyst and ascorbic acid as a reducing agent (Murphy and Riley, 1962). This procedure is subject to arsenate interferences (Chamberlain and Shapiro, 1969), and the acidic conditions of the test may hydrolyze any very labile organic phosphate esters in the sample (Rigler, 1966, 1968). Chamberlain and Shapiro (1973) have concluded, however, that hydrolysis of organic phosphate esters in 0.45 micron pore-size filtered samples would probably not cause significant errors in the assay of soluble orthophosphate in natural surface lake waters.

Another possibility of error would be the presence of acid-labile inorganic phosphate of particulate nature but small enough to pass the 0.45 micron pore-size membrane filters generally employed for soluble orthophosphate analysis. Chamberlain (1968) reported that about 90 percent of the ^{32}P in 0.45 micron pore-size membrane-filtered lake water (prepared from lake water to which soluble inorganic ^{32}P had been added) could be removed by refiltering the water through a 0.01 micron pore-size membrane filter, and about 80 percent of the ^{32}P could be removed by ultracentrifugation.

Some investigators have attempted to evaluate the molybdenum blue procedures by comparison with algal bioassays. Chamberlain and Shapiro (1969) assayed soluble orthophosphate with a 30-second extraction procedure and with a one-hour uptake test with *Microcystis aeruginosa*, and found no serious discrepancies between the results in arsenate-free Minnesota lakes. Kuenzler and Ketchum (1962) and Rigler (1966), however, found discrepancies between the results obtained using isotope partitioning of $^{32}PO_4$ (between algal cells and the growth medium) and the results obtained from chemical analyses of the inorganic P in solution. In Rigler's (1966) case, the discrepancies were 10 to 100 fold, probably as a result of the very low concentrations of soluble inorganic P involved. In contrast, Walton and Lee (1972) found essentially no difference between chemically measured and algal-available orthophosphate in Lake Mendota water, sediment extracts, and algal extracts.

The estimation of available phosphate in runoff soil suspensions is complicated by the presence of significant amounts of inorganic P sorbed to the soil particles. Direct analysis of the runoff by colorimetric procedures for soluble orthophosphate would require a preliminary filtration step, which would prevent the estimation of potentially available inorganic P on the particles filtered off. Since some of this particulate inorganic P may become available to algae through dissolution or desorption reactions in the receiving water, the errors in neglecting soil-bound inorganic P would be quite significant. Agronomists have devised several procedures for estimating the content of plant-available phosphate in soils; these procedures provide data which can be correlated with greenhouse or field tests of plant growth, in order to assess the phosphate status of the soil in terms of agricultural productivity. Various chemical extraction procedures have been found to correlate well with plant growth on various types of soils. Some of the extractants used are water, carbon dioxide saturated water, acids, bases, salts, and various buffered solutions.

Another method of estimating the pool of potentially available inorganic P in soils is radioisotopic exchange with inorganic ^{32}P. Li et al. (1972) found that the amount of inorganic P which would exchange with inorganic ^{32}P comprised from 19 to 43 percent of the total native inorganic P in the sediments. Amer et al. (1955) compared ^{32}P methods to methods where the soil P was adsorbed by anion-exchange resins. Quantities of P adsorbed from soils by resin were less than those quantities which equilibrated with ^{32}P during the same time intervals. However, the correlation coefficient for P adsorbed by the resin in two hours and P-availability to plants in the greenhouse was 0.95.

The ion-exchange resin method has been developed into a practical laboratory procedure for testing soils for available P, through the efforts of Cooke and Hislop (1963) and Hislop and Cooke (1968). They reported that the degree of exchange was temperature dependent, with a two-fold increase in P availability between 10 and 30°C. Time was not a critical factor after 12 hours of equilibration of soil and resin in aqueous suspension (Cooke and Hislop, 1963). Lathwell et al. (1958) compared resin methods with chemical extractive methods. The resin methods gave the highest degree of correlation with crop response data, especially with respect to phosphorus uptake by plants. Kunishi et al. (1972) used the method of Hislop and Cooke (1968) to estimate the available P sorbed by eroded soils from an agricultural drainage basin, during actual runoff events. About 5 to 20 percent of the amount adsorbed remained available to the resin.

In contrast to the soil P availability tests which are based on correlations with plant growth or phosphorus uptake, other tests have been developed for the purpose of quantitating the chemical forms of soil P. These tests contribute to an understanding of the processes by which phosphorus in soils is fixed or released. One of the earliest attempts at a scheme of soil phosphorus fractionation was that of Chang and Jackson (1957), who classified phosphates in soils into four main groups: calcium phosphate, aluminum phosphate, iron phosphate, and reductant-soluble phosphate extractable after removal of the first three forms. Their extraction scheme employed successive extractions of the soil with ammonium fluoride (0.5N), sodium hydroxide (0.1N), sulfuric acid (0.5N), and sodium citrate-sodium dithionite solution. Each extractant was intended to measure one of the four main groups of phosphate when the extractions were carried out in the proper sequence. However, their characterization of iron, aluminum, and calcium phosphates as discrete chemical forms probably is not entirely accurate because of the presence of amorphous, poorly soluble phosphates resulting from sorption or precipitation reactions on soil surfaces (Stumm and Morgan, 1970).

Several modifications of the Chang and Jackson (1957) methods have been proposed in recent years. Williams et al.

(1971b) found that with calcareous lake sediments the Chang and Jackson procedure underestimated the total amount of inorganic P solubilized during the ammonium fluoride and sodium hydroxide extractions, because of resorption of the inorganic P by calcium carbonate or calcium fluoride. They proposed a fractionation scheme for calcareous soils based on successive single extractions with sodium hydroxide, citrate-dithionite-bicarbonate, and hydrochloric acid. Shukla *et al*. (1971) used both acid ammonium oxalate and citrate-dithionite-bicarbonate to show that sorption of inorganic P was apparently controlled by amorphous iron oxides in both calcareous and non-calcareous lake sediments.

In summary, the soil P availability tests developed for routine use are operationally defined, although they may be correlated with soil P availability measured biologically. The procedures for measuring the various forms of phosphorus in soils may also be related to biological availability, if the P form being measured is the same form responsible for phosphorus release under the proper conditions. It is clear that in both types of tests, some correlative relationship to plant (or algal, in the case of lake sediments) bioassays must exist if the tests are to have practical uses.

Biological Methods

Generally, the chemical methods of measuring available phosphorus measure dissolved inorganic P plus inorganic P extracted from soils or other insoluble particles. The contribution from organic P forms in solution or in the soils are neglected as insignificant compared to inorganic P. This is probably a valid assumption in the case of runoff or river waters carrying large suspended soil loads. In cases where much of the phosphorus may also exist as vegetal matter or as microbial cells (bacteria, algae, and zooplankton), biological techniques are needed. Three basic techniques have been used: batch mineralization systems, cell-free systems, and algal bioassays.

Waksman et al. (1937) used batch mineralization systems to estimate the extent of nutrient regeneration in sea water. Samples of water were stored in darkness to allow bacterial growth and subsequent die-off. During incubation, inorganic P in solution was measured in order to follow the release of available P with time. Renn (1937) conducted similar experiments and demonstrated that P assimilation occurred during the first few days of the test, when the bacterial numbers were increasing to 10^3 to 10^5 times their normal population levels in natural sea water. P regeneration followed rapidly after die-off of the bacteria. When a carbon source such as glucose was added, the time required for P regeneration was much longer than in normal sea water. Consequently, the efficiency of bacteria as agents of phosphorus regeneration is related to the supply of other nutrients besides phosphorus in the water. If these nutrients are in short supply relative to phosphorus, P regeneration will be rapid, as the life span of the bacteria will be short due to the nutrient limitations.

If chloroform or toluene is added to soils or natural waters, the microbial cells will lyse and release their cellular phosphates and phosphatase enzymes into solution. This cell-free method results in a much more rapid rate of inorganic P production than in batch mineralization systems, since microbial P assimilation reactions are prevented by the sterilizing chemicals. Thompson and Black (1949) showed a three-fold increase in the apparent hydrolysis of soil organic P in toluene-treated samples compared to untreated controls. Berman (1970) incubated Lake Kinneret water with chloroform to show the release of soluble inorganic P from the dissolved organic or particulate P in the lake. About 53 percent of the total P in the samples was converted to dissolved reactive P in three to five days. However, his samples probably contained mainly algal-P and zooplankton-P, hence his results would not be expected to be characteristic of an urban runoff system with large suspended soil loads. A similar study by Golterman (1960) showed that almost 65 percent of the total cell P was released as dissolved reactive P in a 28-day incubation of chloroform-treated _Scenedesmus_ cells.

In the presence of soils, any inorganic P released via cell lysis or phosphatase enzyme action may be sorbed by the soils and never measured in solution. Acid extractions have been used by several workers to remove the sorbed inorganic P. Rogers (1942) used 10 percent hydrochloric acid, and Thompson and Black (1949) and Bower (1949) used 1N sulfuric acid in tests with toluene.

Direct algal bioassay of available P in soils has been performed by Fitzgerald (1970a), who incubated P-starved _Cladophora_ in direct contact with aerobic lake muds. The (filamentous) alga was then separated from the mud for cellular P analysis. Boiling water extractions of the cells showed that, at most, only one percent of the total sediment P was available. Golterman _et al_. (1969), in contrast, demonstrated that the phosphate taken up from their lake muds by _Scenedesmus_ was about 5 to 30 percent of the total P in the muds. Similarly, Spear (1970) conducted long-term dark aerobic incubations of muds from the same lake (Mendota) sampled by Fitzgerald, and found phosphorus release. Tests of phosphorus precipitated by iron or calcium in systems where unicellular _Chlorella_ was separated from the insoluble P forms by dialysis tubing or membranes showed that such P sources were available for algal growth (Fitzgerald, 1970a,b). This was also demonstrated by Golterman *et al*. (1969), with *Scenedesmus* grown in direct contact with $FePO_4$ (added in dry form) or hydroxyapatite. Apparently the mechanisms governing P availability in lake muds are not explained by simple insolubility properties alone.

The direct algal bioassay of filtered waters has been used by Skulberg (1964) and Baalsrud (1967) in Europe. Their work eventually led to the Algal Assay Procedure (AAP) Bottle Test (Environmental Protection Agency, 1971). This procedure called for filtration of the water sample before inoculation with the test alga, although the sample could be autoclaved prior to the filtration if an estimate of the available P in bacterial, algal, and zooplankton cells were desired. The filtration step was required in order to eliminate competition between the natural organisms and the inoculum (test) alga and to eliminate interferences in the measurement of algal growth by light absorbance or fluorescence procedures. Such interferences would be caused by detritus or soil particles in the natural water samples. Golterman *et al*. (1969) were able to quantitate *Scenedesmus obliquus* cells in the presence of lake muds by making microscopic cell counts, thus eliminating the need to alter the natural system by filtering or autoclaving. Another way to avoid such alterations has been demonstrated by Goldman *et al*. (1969), who measured the rate of carbon-14 dioxide uptake by natural plankton populations in unfiltered samples. They reported this method to be more sensitive than cell counts for the quantitation of algal population changes. The carbon-14 procedure was also used by Plumb (1973) in measurements of the response of very dilute populations of Lake Superior phytoplankton to taconite tailings.

SUMMARY

A review of the literature has indicated that urban runoff carries a high concentration of phosphorus, much of which is in the particulate phase. The fraction of the particulate P which might be available for algal growth has not been thoroughly investigated, however, either in the runoff or in river waters containing a combination of runoff and wastewater inputs.

SECTION V

SAMPLING OF URBAN RUNOFF AND LAKE ONTARIO TRIBUTARIES

MADISON URBAN RUNOFF

Samples of urban runoff were collected from several sites in the city of Madison, Wisconsin (population 171,769 in 1970), from August, 1972 to March, 1973 (Table 7.) Table 8 lists the sampling stations and their locations. The type of urban land use in the vicinity of each station is given in Table 9 . Three major types of land use were selected: residential, commercial, and urban construction. Runoff from industrial land uses was not sampled, because such areas constituted a small fraction of the total Madison urban area. Although construction sites also account for a small portion of the urban area, the high yields of sediment from urban construction activities (Wolman and Schick, 1967; Thompson, 1970) likely contribute to appreciable phosphorus transport.

Residential population densities in Madison generally decrease along a line from the state capitol square (see Figure 2 ; on the isthmus between Lakes Mendota and Monona) to the western boundary of the city. Thus, the locations of sampling stations H, D, B, and A shown in Figure 2 were in areas zoned R6, R5, R2, and R1, areas of decreasing population density, respectively (see Table 9). In terms of forestry, the area around station B on

Table 7. MADISON STORMWATER RUNOFF RECORD

Runoff Event No.	Date
1	August 11, 1972
2	August 19, 1972
3	August 23, 1972
4	September 19, 1972
5	September 20, 1972
6	October 20, 1972
7	October 22, 1972
8	December 30, 1972
9*	January 17, 1973
10	January 18, 1973
11	February 1, 1973
12	March 5, 1973

*Runoff was from snowmelt alone, with no precipitation.

Table 8. MADISON URBAN RUNOFF SAMPLING STATIONS

Station	Location
A	Inlet grate of the open storm sewer in the median strip of Whitney Way, near the Montauk Place Intersection
B	Inlet grate of the open storm sewer in the median strip of Manitou Way, near the Tumalo Trail intersection
D	Outlet of the storm drain pipe under the Water Chemistry Laboratory driveway, on the U. of Wis. campus
E	Street gutter inlet grate at the intersection of King, Butler, and Wilson Streets; on Wilson Street
F	Street gutter near the corner of Island Drive and Masthead Streets; on Island Drive
G	Outlet of the large storm sewer pipe in Law Park which drains the capitol square area and is located on line with Pinckney Street extended
H	Street gutter inlet grate at the end of Broom Street near the intersection of Broom Street and John Nolan Drive
I	Street gutter on Whitney Way, near the Sheboygan Street Intersection

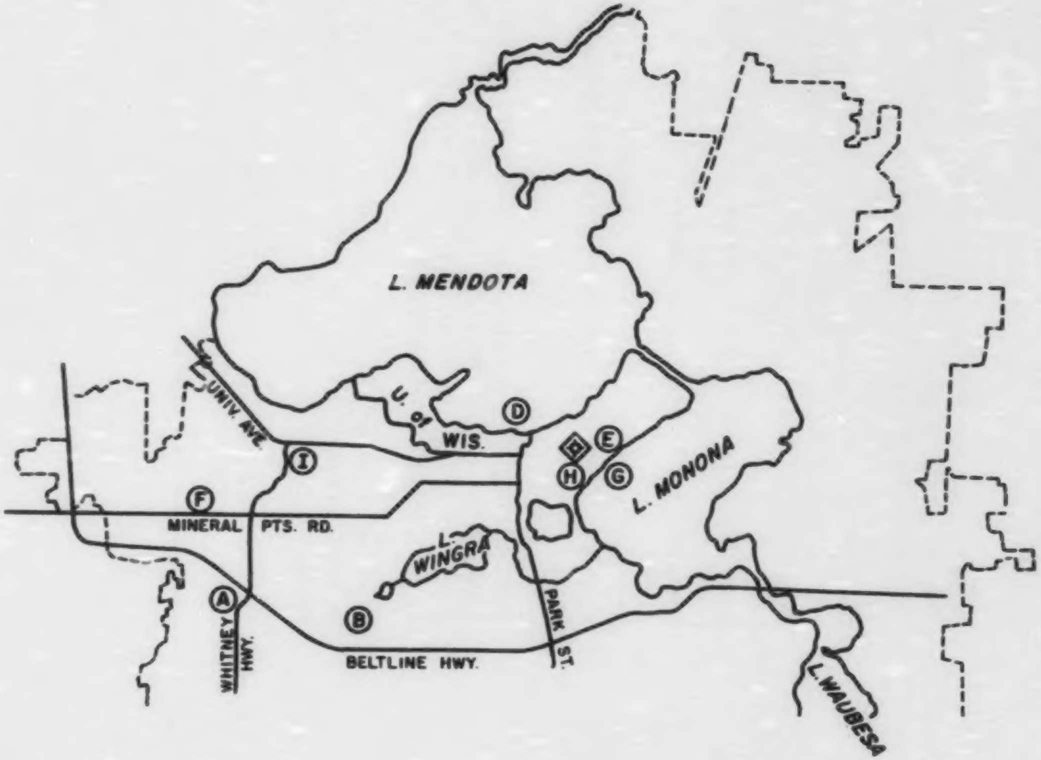

L. MENDOTA
U. of WIS.
D
E
H
G
L. MONONA
UNIV. AVE.
I
F
MINERAL PTS. RD.
L. WINGRA
A
WHITNEY HWY.
B
BELTLINE HWY.
PARK ST.
L. WAUBESA

Figure 2. Urban runoff sampling stations in Madison, Wisconsin

Table 9. DESCRIPTION OF THE URBAN AREAS SAMPLED FOR RUNOFF PHOSPHORUS

Sampling Station	Madison Zoning Code for Drainage Area	Description of Land Uses in the Drainage Area
A	R1	A single family residential area, with some low density multiple family dwellings
B	R2	An area much like R1, except that less useable open space per dwelling is allowed
D	R5	The University of Wisconsin campus area around Bascom Hill
H	R6	A high density residential area located near the center of the city, with low useable open space allowed per dwelling unit
E and G	C4	The central commercial district of Madison, around the capitol square
F	R1, R5, C1 and C2	A residential-light commercial area; construction activity with soil erosion until sod was put down
I	R2	A residential area; construction activity with soil erosion

Manitou Way appeared to have the highest density of trees of all the residential stations. The lowest density of trees was in the area near Station H (Broom Street).

The commercial area selected comprised the central business district of Madison, located around the capitol square. This section of the city is characterized by heavy automobile and pedestrian traffic, and most of the land area is covered by impervious (street, sidewalk, and roof) surfaces. Two stations were selected for collection of runoff from the commercial area (Table 9). Station G was sampled one time, on September 20, 1972; all other samples were collected from Station E which could be sampled more conveniently than Station G.

Madison urban construction sites were chosen for sampling on the basis of observed soil erosion during runoff events in the summer of 1972. Station F was sampled from August, 1972 to January, 1973. The last sample taken, January 17, was taken after the site had been sodded to prevent further erosion. Hence, this sample (No. F-9) probably represented the runoff from a residential-light commercial area more closely than runoff from a construction site. Station I was selected at a construction site about one mile from Station F, as an alternate site for samples of construction site runoff collected after January 17, 1973.

All runoff samples were collected as grab samples in one-gallon acid-washed polyethylene cubitainers. Shallow streams of runoff in street gutters were sampled with a partially collapsed cubitainer, so that the mouth of the container could be placed as close to the gutter surface as possible. Repeated grab samples collected in this manner were combined in a second cubitainer until a gallon of sample water was collected. Most samples were collected at inlet grates, where the runoff was falling over a ledge and could be readily caught in a cubitainer. Because the depths of the runoff streams were usually quite shallow and the flows fairly turbulent, the particulate matter in the samples was assumed to be representative of the particle size distribution being transported in the runoff. Observation of samples allowed to stand a short time after collection often showed the presence of relatively heavy sediment, indicating that the sampling procedures were probably not introducing a serious bias by collecting only light particulate matter.

Previous urban runoff studies (Kluesener, 1971; Weibel _et al_., 1964) have shown that grab samples taken at different times during the runoff event may vary both in the phosphorus concentration and in the ratio of soluble to particulate P forms. The size distribution of the particulate matter transported might also be expected to vary, and this could cause a qualitative change in the nature of the particulate P forms in the samples, hence also affect the measured phosphorus availability of particulate P forms. It was hoped that by sampling a given site more than once over a relatively long period of time, some of these differences in particle size distribution might be taken into account in an overall average availability value for that site.

PRECIPITATION SAMPLES

In an effort to assess the algal growth potential of dustfall and precipitation, snow samples were collected in Madison and rain gage samples were received from IFYGL personnel in the state of New York.

Madison snow samples were collected from the following three sites on April 10, 1973: 1) the roof of the City-County Building near the state capitol, 2) a lawn across

Commercial Avenue from the Oscar Mayer meat packing plant, and 3) a hill just inside the entrance to the Picnic Point Park on the University of Wisconsin campus. Surface layers of snow 0 to 3 cm in depth were scraped into plastic bottles which had been cut to form scoops. The scoop-bottles were covered with aluminum foil and stored at 4°C until analysis.

New York rain gages were established by the Environmental Protection Agency (EPA) Laboratory, Rochester, N.Y., at the locations shown in Table 10. The notations "open" or "closed" in the table indicate the collection of bulk precipitation (dustfall plus rainfall) or of rainfall alone, respectively. Samples were collected during May and June, 1973, and were shipped to Madison in plastic bottles without addition of preservatives.

GENESEE RIVER BASIN SAMPLES

In cooperation with the New York State Department of Environmental Conservation, water samples from streams in the Genesee River Basin were sent to Madison for analyses of phosphorus availability. Table 11 lists the locations of the sampling stations along with the predominant land use in the sub-basin drained by each stream. A detailed description of land uses within the sub-basins, expressed in terms of acreage and percent of total area, is given in Table 12. The sub-basins are listed according to predominant land use. Two of the stations were located in urban areas, while the others were located in agricultural, forested, or brushland areas. All samples were collected and sent to Madison in one-gallon cubitainers enclosed in an insulated box with two to three small bottles of frozen water for refrigeration. The samples were generally collected on a biweekly schedule and were received in Madison 1 to 9 days after collection.

Table 10. LOCATION OF THE EPA NEW YORK RAIN GAGES

Sample No.*	Precipitation Collection Period	Rain Gage Location
601-O	May 1 - May 30, 1973	Macedon, N.Y.
602-C	May 1 - May 30, 1973	Skeneateles, N.Y.
603-C	May 1 - May 30, 1973	Oswego, N.Y.
604-C	May 1 - May 30, 1973	Brockport, N.Y.
605-C	May 1 - May 30, 1973	University of Rochester, N.Y.
606-C	May 1 - May 30, 1973	Cape Vincent,N.Y.
601-C	June 1 - June 30, 1973	Macedon, N.Y.
602-C	June 1 - June 30, 1973	Skeneateles, N.Y.
603-O	June 1 - June 30, 1973	Oswego, N.Y.
604-C	June 1 - June 30, 1973	Brockport, N.Y.
605-C	June 1 - June 30, 1973	University of Rochester, N.Y.
606-C	June 1 - June 30, 1973	Cape Vincent,N.Y.
608-O	June 1 - June 30, 1973	Clarence, N.Y.

* O = Open at all times
C = Closed except during precipitation events
(Source: Casey, 1973)

Table 11. LOCATION OF RUNOFF SAMPLING STATIONS IN THE GENESEE RIVER BASIN, N. Y.

Station No.	Station Location, U.S.G.S.* Map Name	Predominant Land Use	Stream Sampled
1	Bryon	Cropland	Spring Creek
2	Rochester East	Urban	Allen Creek
3	Geneseo	Beginning of pasture	--
4	Geneseo	Pasture	Jaycox Creek
5	Geneseo	Beginning of pasture	--
6	Springwater	Forest	Briggs Gully
7	Dansville	High density residential	--
8	Dansville	Beginning of high density residential	--
9	Andover	Brushland	East Valley Creek

*United States Geological Survey

(Source: Boulton, 1972)

Table 12. LAND USE DISTRIBUTION IN SUB-BASINS OF THE GENESEE RIVER BASIN, N. Y.

Land Use	No. Acres	% of Total Area
Cropland - Spring Creek (Batavia N. and Bryon)		
Cropland	7063	57
Brush	1046	8
Forest	720	6
Bogs and Wooded Wetlands	761	6
High intensity agriculture	2047	16
Pasture	739	6
Misc.	157	1
TOTAL	12533	100
Pasture - Jaycox Creek (Geneseo)		
Pasture	1168	95
Forest	8	1
Brush	31	3
Agriculture - inactive	16	1
TOTAL	1223	100
Brushland - East Valley Creek (Andover and Greenwood)		
Brush	2571	53
Forest	788	16
Agriculture	1409	30
Misc.	36	1
TOTAL	4804	100
Forest - Briggs Gully (Springwater and Bristol Springs)		
Forest	2016	71
Brush	622	22
Cropland	20	1
Bogs and Wooded Wetland	179	6
Misc.	2	0
TOTAL	2839	100

Table 12. (continued)

LAND USE DISTRIBUTION IN SUB-BASINS
OF THE GENESEE RIVER BASIN, N.Y.

Land Use	No. Acres	% of Total Area
	Urban - Allen Creek (Rochester E. and Mendon Ponds)	
Residential, Public Outdoor recreation Commercial	8366	52
Agriculture	3771	23
Forest and Brush	3194	19
Industrial	288	2
Misc.	646	4
TOTAL	16265	100
	Residential (High Density) (Dansville)	
Residential (high density)	159	91
Urban (downtown)	15	9
TOTAL	174	100

(Source: Boulton, 1972)

LAKE ONTARIO TRIBUTARY SAMPLES

Four New York tributaries to Lake Ontario were selected for sampling (Figure 1 arrows). Grab samples were collected periodically to dtudy the forms of phosphorus carried in suspension by the rivers. No attempt was made to sample the bed load (sediment transported along the stream bottom). Several samples of river water were collected by personnel of the State Universities of New York at Buffalo and Oswego. Other samples were collected by University of Wisconsin students and staff during the 1973 period of high spring flow at the following locations:

The Niagara River was sampled at two locations, one above Niagara Falls at Beaver Island State Park (Grand Island), and one below the falls at the mouth of the river, near the Fort Niagara Coast Guard Station on Lake Ontario. The Beaver Island Park samples were taken from the west branch of the river, not far from the southern tip of Grand Island. The Fort Niagara samples were taken from the New York bank of the river, about 50 yards from the mouth.

The Genesee River was sampled from the Route 104 bridge, or from the navigation locks near the bridge, in the city of Oswego. The Route 104 bridge is located less than a mile from the river mouth.

The Black River was sampled from the south bank near the Route 180 bridge at Dexter, New York, which is located about one mile from Black River Bay on Lake Ontario.

The spring flow samples were collected from the surface of the rivers with a plastic bucket, which was either lowered from a bridge or thrown out from shore. All samples were shipped to Madison on commercial airlines; samples sent by workers in New York were receoved 4 to 9 days after collection, while samples collected by University of Wisconsin students and staff were received within two days of collection. All samples were stored at 4°C until analyzed; generally, such storage lasted 2 to 7 days.

SECTION VI

ANALYTICAL METHODS

All water samples in this investigation were analyzed by chemical and biological methods designed to estimate the algal-available fraction of the particulate P, soluble P, or total P in the samples. This chapter presents the methods used to quantitate the phosphorus forms and to estimate their availability. The rationale for the methods and an evaluation of them is also presented.

CHEMICAL ANALYSES OF PHOSPHORUS FORMS

Useful estimates of phosphorus availability in natural waters may be obtained from chemical analyses of phosphorus forms where such analyses have been correlated with the results of algal growth bioassays, as in the studies of Walton and Lee (1972), and Chamberlain and Shapiro (1969). Chemical methods serve to qualify total phosphorus measurements by providing estimates of the fraction of total P available to algae.

In this investigation, the following seven operationally defined phosphorus forms or fractions were selected for analysis:

1) Dissolved reactive phosphorus (DRP)
2) Total soluble phosphorus (TSP)
3) Particulate phosphorus (PP)
4) Total phosphorus (TP)

5) Particulate inorganic phosphorus (PP_i) extracted by:

   a) Acid

   b) Base

   c) Anion-exchange resin

The first four forms in the above list were determined to quantitate the phosphorus forms in the samples. TP gives the upper limit of available P. DRP includes mainly dissolved orthophosphate and hence gives an estimate of the readily available P. TSP includes DRP plus other soluble, less readily available forms such as condensed phosphates or organic phosphate esters. PP consists of the total inorganic plus organic phosphate associated with particulate matter; hence PP is less readily available than DRP.

The PP extraction procedures were selected from the literature (Wentz, 1967; Williams et al., 1967; and Hislop and Cooke, 1968) to approximate the fraction of PP which might be available to algae in a body of water. Although these procedures may extract both organic and inorganic P forms from the PP, the analytical methods used to determine the quantity of P in the extract measures mainly inorganic P. Consequently, the chemically measured P extracted from PP is termed the "PP_i extracted" by acid, base, or resin.

PP_i extracted by acid includes calcium phosphates and much of the iron- and aluminum-bound phosphates. PP_i extracted by base includes phosphates bound to iron or aluminum but not those associated with calcium. Neither method measures phosphates occluded in oxides of iron (reductant-soluble P). Consequently, the phosphates measured are probably those precipitated or adsorbed on the surfaces of soil particles or bound in discrete mineral phases. If surface-bound phosphate is the major fraction extracted, then the extractable PP_i should be correlated with available P. Surface-bound phosphates should be more readily available than occluded or slowly-soluble discrete phase phosphates (Chang and Jackson, 1957). Since neither the acid nor the base extractions should cause appreciable hydrolysis of organic phosphates, the PP_i in the extracts should be primarily derived from inorganic P forms associated with particulate matter.

The PP_i extracted by resin is comprised of that inorganic P on the particles which can equilibrate fairly rapidly with the inorganic P in the solution phase around the particles. By decreasing the concentration of inorganic P in the solution phase to low levels, the anion-exchange resin forces a reversal of phosphate sorption and precipitation reactions. The PP_i transported to the resin "sink" is expected to approximate the inorganic phosphate which would be released from the particles upon dilution in a phosphorus-deficient receiving water.

Dissolved Reactive Phosphorus

DRP was determined by the colorimetric method of Murphy and Riley (1962) after filtration of the samples to remove particulate matter. The color reagent was added to the filtrates in the ratio of 3 ml of reagent to 40 ml of filtrate, and the mixture was diluted to 50 ml, as specified by Murphy and Riley (1962). Alternatively, 4 ml of color reagent was added to 20 ml of sample without dilution. In either case, standards, blanks, and samples were treated alike to compensate for the different volume ratios used.

Filtration for the initial DRP values of all samples was performed with 0.45 micron pore-size Millipore[a] membrane filters, which had been presoaked in dilute HCl (1 to 2 ml concentrated HCl per 500 ml of water) for at least one day before use. The filters were rinsed and stored in distilled water until used. Chemical extracts of PP or dark-incubated water samples were filtered through either membrane or No. 934AH Reeve-Angel glass fiber filters[a], of undefined pore size. The objective of filtration was to obtain a clarified sample for the colorimetric analysis of soluble inorganic P. In most cases, glass fiber filters were used for river waters and membrane filters for urban runoff. After December, 1972, however, all filtrations were performed with membrane filters. Table 13 shows the effect of filter type on the colorimetric analysis of New York river waters. Significant differences between the results with glass fiber and membrane filters at the 95 percent confidence level were found for two of the samples tested, while only one sample (No. 42) showed a significant difference at the 99 percent confidence level. In terms of the fraction of total P represented by the two mean values for sample No. 42, however, the difference was only 2 percent (23 and 21 percent of total P for glass fiber and membrane filter methods, respectively).

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[a] Millipore Corp., Bedford, Mass.

Because of the wide range of phosphorus concentrations encountered in these investigations, contamination of volumetric glassware, especially pipettes, was considered a potential problem. To minimize this problem, the use of pipettes was restricted by using calibrated test tubes for the measurement of filtrate volumes and as vessels for performing the DRP color reaction on the filtrates. Since the color reagent effectively "cleaned" the test tubes by a complexation reaction

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[a] Reeve Angel Co., Clifton, N.J.

Table 13. EFFECT OF FILTER TYPE ON THE DRP ANALYSIS OF NEW YORK RIVER WATERS

Sample		DRP Concentration in Filtrate (µgP/l)	
		No. 934AH Reeve Angel glass fiber filters	0.45 micron pore size Millipore filters
No. 40 Niagara R.		3	3
		3	3
		5	3
		5	3
		3	3
	mean value	4[a]	3[a]
	std. deviation	0.7	0
No. 42 Genesee R.		33	31
		35	31
		35	31
		35	32
		35	31
	mean value	35[a,b]	31[a,b]
	std. deviation	0.9	0.4
No. 44 Black R.		6	6
		6	6
		6	6
		6	6
		6	6
	mean value	6	6
	std. deviation	0	0
No. 47 Oswego R.		57	55
		55	57
		57	57
		61	57
		--	57
	mean value	58	57
	std. deviation	6.5	0.9

[a] The mean values for glass fiber and Millipore filters were significantly different at the 95% confidence level.

[b] The mean values for glass fiber and Millipore filters were significantly different at the 99% confidence level.

with the phosphate, only a few rinses with distilled water were required to wash the tubes for the next set of samples. A set of 25 X 200 mm test tubes was calibrated at 20, 40, and 50 ml volumes by pipetting water into the tubes and marking the meniscus with a black line on the outside of the tubes. Table 14 presents the results of replicate DRP analyses made by filling ten randomly selected tubes to the 20 ml marks with a river water filtrate. Four ml of Murphy and Riley (1962) reagent were added to each tube with a reproducible-volume Repipet[a] so that the results of the analyses would reflect the variability of the sample measurement step. The coefficient of variation was only 1 percent in this test. Volumetric measurement of 40 ml with the calibrated test tubes would have even less variability because the relative volume error would be halved in comparison to measurement of 20 ml volumes.

The absorbance of the color resulting from addition of the Murphy and Riley reagent to 20 or 40 ml of sample was measured after about 30 minutes on a Beckman Model DU spectrophotometer[b] with either a 5 or 10 cm absorption cell, or on a Spectronic 20[c] spectrophotometer with either a ½-inch or 1-inch absorption cell. Each set of samples was accompanied by a series of standards and a reagent blank, plus a membrane filter bank as necessary. All blanks, samples, and standards were measured against distilled water at 882 nm wavelength. The standard solutions were prepared with volumetric glassware.

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[a] Labindustries Co., Berkeley, Cal.
[b] Beckman Instruments, Inc., Fullerton, Cal.
[c] Bausch and Lomb Co., Rochester, N.Y.

Table 14. PRECISION OF DRP ANALYSES USING MARKED TEST TUBES

Sample Replication No.		DRP (ugP/l)
1		60
2		62
3		62
4		62
5		62
6		62
7		62
8		62
9		62
10		62
	mean value	62
	standard deviation	0.6
	coefficient of variation	1%

Total Soluble Phosphorus

TSP was determined in the same filtrates used for DRP analysis. A 20 ml sample was transferred into a calibrated test tube or pipetted into a 50 ml Erlenmeyer flask, and 0.2 ml of a strong sulfuric acid solution (30 ml of concentrated H_2SO_4 diluted to 100 ml with water) added. A freshly prepared solution of potassium persulfate was made by adding 5 grams of the salt to 100 ml of water and warming on a hot plate to just dissolve the salt. A 3 ml aliquot of this solution was added to each acidified sample. The flasks or digestion tubes were capped with aluminum foil and autoclaved for about one hour at 15 pounds per square inch (psi) pressure. The samples were then allowed to cool, and a predetermined volume of 3N NaOH was added to neutralize the digestion acid. DRP was determined after dilution of the mixture to 50 ml with water and 8 ml of color reagent.

Total Phosphorus

TP was determined in unfiltered samples using a digestion procedure similar to that used for TSP, except that a filtration step was sometimes required after the digestion. Residual particulate matter was filtered out with glass fiber or membrane filters before neutralization of the samples with 3N NaOH. If neutralization preceded filtration, much of the phosphorus was lost in the filtration step. Apparently iron hydroxides were formed at the neutral pH and were responsible for sorption of phosphate during the subsequent filtration. Standards and blanks prepared for the TSP analysis and carried through the persulfate digestion procedure described above were also used to quantitate TP. All volumetric measurements of samples to be analyzed for TP were made with graduated cylinders in an effort to obtain representative suspensions of particulate matter.

Particulate Phosphorus

PP, the total insoluble inorganic and organic phosphorus in a sample, was determined either by calculation (TP-TSP) or by direct persulfate digestion of particles separated from the bulk solution by membrane filtration.

Analysis by calculation was performed by subtracting the mean value of three replicate TSP determinations from the mean value of three replicate TP determinations made on the same sample. The results obtained in this manner were generally used to quantitate the amount of particulate phosphorus taken for chemical extraction. In these extractions, the sample particulate matter was deposited on a membrane filter by passing the sample through the filter. The filter plus particles were then placed into extraction solution and scraped clean with a metal spatula. Since the filters were soaked for at least 5 minutes, even fine material retained in the pores of the filter may have been extracted to some extent. As an approximation, it was assumed that all of the PP deposited on the filter ((TP-TSP) X volume filtered) from the sample was subjected to extraction, so the PP_i found in the extract could be expressed as a percent of the calculated PP on the filter.

Particulate matter for algal bioassays was isolated from the bulk solution in the same manner as described above. The particles deposited on the filters (non-filtrable particles) were scraped into P-free algal medium, and the filters were removed from the medium before inoculation with algae. Thus, some of the sample particles may have been trapped in the filters and hence not subjected to the bioassay. The true concentration of PP in the algal medium would then differ from the concentration computed by difference (TP-TSP). The true concentration was best estimated in this case by performing a TP (persulfate digestion) analysis on the suspension of particles in the medium or on a similarly prepared suspension in distilled water.

Table 15 presents a comparison of the two procedures for estimating the total PP in a sample. A "scraping efficiency" ratio was computed as the ratio of PP found by digestion of scraped particles divided by PP determined from calculation (TP-TSP). An overall average ratio of 0.95 was found from the 19 samples tested. Low values (<0.80) were seen in three samples. Two of the samples, No. 50 and No. 56 had very low PP values, and PP determined by calculation may have been subject to a relatively large error (Appendix A). The low value for the other sample (No. 59) may have been due to an experimental error in the recorded volume of sample filtered to obtain the particles for direct digestion, as this volume was required along with the analytical concentration of PP found by direct digestion, to compute the value shown in column two of Table 15 (59 μgP/1). In general, the results shown in this table indicate that most of the particulate phosphorus in the bulk sample was transferred to the bioassay medium or into the chemical extraction solutions.

The terms TP, TSP, and (total) PP, which have been used in connection with persulfate digestions, were operationally defined by that digestion procedure. The

Table 15. EFFICIENCY OF SCRAPING PARTICULATE MATTER FROM MEMBRANE FILTERS

Sample	Sample PP (µgP/l)[a] (1) Determined from scraped particles[b]	(2) Determined by calculation (TP-TSP)	Ratio, (1)/(2)
	Madison Runoff		
E-11	138	145	0.95
D-11	453	441	1.03
H-11	253	262	0.96
A-12	194	214	0.91
B-12	408	421	0.97
D-12	524	561	0.93
I-12	1540	1419	1.09
	New York Rivers		
Genesee R.			
No. 34	387	360	1.08
No. 42	101	105	0.96
No. 51	62	62	1.00
No. 58	128	150	0.86
Oswego R.			
No. 43	56	50	1.12
No. 52	51	48	1.06
No. 59	59	88	0.67
Black R.			
No. 44	24	19	1.26
No. 53	24	25	0.96
No. 60	68	75	0.91
Niagara R.			
No. 50	12	19	0.63
No. 56	13	26	0.50
		Average	0.95

---

[a] Mean values of triplicate determinations of PP, or of TP and TSP used in the calculation (TP-TSP)

[b] Values obtained from direct persulfate digestion of particles in distilled water or (-P) algal medium of volume v, where the particles were derived from a volume V of original sample. Thus:

$$\mathrm{PP}_{(\text{in table})} = \mathrm{PP}_{(\text{from digestion})} \mathrm{X}\ (v/V)$$

persulfate digestion procedure used routinely in the studies reported here was essentially that given in Standard Methods (1971). Although it was realized that this procedure may not have measured all of the phosphorus in some types of samples, the method was used because of its wide usage in other studies of urban runoff and nutrient budgets for lakes, as well as in related International Field Year for the Great Lakes (IFYGL) research. The calculated values of percent availability may be applicable to other workers' TP or PP data, if all the data are based on a common digestion procedure.

Table 16 shows that the persulfate method was not significantly less effective than a method using a stronger oxidizing agent (perchloric acid in a ternary mixture with nitric and sulfuric acids(for one of the samples tested (urban runoff No. D-12). For the other sample tested (No. I-12, collected near an urban construction site), the perchloric acid method gave a TP value about 8 percent higher than the TP value from the persulfate procedure. With a solution of inositol phosphates supplied by Dr. W. Weimer[a], both methods appeared to be equally effective. In contrast to the oxidizing methods, a simple hot (one hour at 100°C) acid hydrolysis with the sulfuric acid used in the persulfate method was very ineffective in a TSP analysis of the inositol phosphates. Based on the tests reported in Table 16 , the persulfate method probably underestimates TP, TSP, or PP by 8 percent or less relative to the perchloric acid method.

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[a] Dr. Walter C. Weimer was a graduate student at the University of Wisconsin Water Chemistry Laboratory at the time of these investigations. His present address is Pacific Northwest Laboratories, Battelle Boulevard, Richland, Wash. 99342.

Table 16. EFFICIENCY OF THE PERSULFATE DIGESTION METHOD AS COMPARED TO A PERCHLORIC ACID METHOD

1.) Particles Filtered from Madison Runoff

	PP (µgP/l)			
Sample No.	Persulfate digestion[b]		Perchloric acid digestion[b]	
D-12	145		175	
	152		130	
	150		145	
	145		177	
	152		140	
mean values $\bar{x}_1$ =	149	$\bar{x}_2$ =	153	
std. deviation s_1 =	4	s_2 =	21	

$(\bar{x}_2 - \bar{x}_1)$ not significant at the 95% confidence level

I-12	410		407
	405		455
	400		455
	395		407
	400		460
mean values $\bar{x}_1$ =	402	$\bar{x}_2$ =	437
std. deviation s_1 =	6	s_2 =	27

$(\bar{x}_2 - \bar{x}_1)$ significant at the 95% confidence level

$(\bar{x}_2 - \bar{x}_1)$ = 8 percent of $\bar{x}_2$

2.) Inositol Phosphate Solution

A. Persulfate Digestion[a]

Replicate No.	TSP (µgP/l)
1	398
2	398
3	400
4	398
range	398-400

Table 16 (cont'd). EFFICIENCY OF THE PERSULFATE DIGESTION METHOD AS COMPARED TO A PERCHLORIC ACID METHOD

B. Perchloric Acid Digestion[b]

Replicate No.	TSP (μgP/l)
1	385
2	402
range	385-402

C. Sulfuric Acid[c]

TSP - 18 μgP/l

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[a] Method given in text.

[b] 20 ml sample + 1 ml conc. $HNO_3/HCLO_4$(60%)/conc. H_2SO_4 predigestion of 20 ml sample W/1 ml conc. HNO_3.

[c] 20 ml sample + 0.2 ml of H_2SO_4 soln. given in text; heated 1 hr. at 100°C.

Table 17 summarizes the estimated random errors of each of the analytical methods used to characterize the phosphorus forms in runoff and stream samples. The statistical methods used in these estimates are given in Appendix A. Coefficients of variation (cv) of 5.5 percent or less were found for the methods of direct analysis. PP determined by calculation as TP-TSP would have a cv which would be partially dependent upon the value of PP. If this value were about the same as ($\geq$ 90 percent of) TP, the cv for PP would be about 2 percent, as calculated using the cv values for TP and TSP in Table 17. The cv for PP would be increased to 4 and 9 percent of PP if PP were decreased to one-half and one-fourth of TP, respectively. The approximate accuracy of the analyses was tested by adding orthophosphate spikes to samples used for DRP or TP analyses (Table 18). The average recovery of a 25 μgP/l spike in the DRP analyses was 23 μgP.l, or 92 percent. The same percent spike recovery was found for a 500 μgP/l spike in a TP analysis of urban runoff Sample B-9.

Table 17. ESTIMATED PRECISION OF CHEMICAL ANALYSES FOR SOLUBLE AND PARTICULATE PHOSPHORUS FORMS

Procedure	Number of Replicates	Mean Value	Range	Standard Deviation	Coefficient of Variation
		(μgP/l)			
DRP	10	62	60-62	0.6	1.0%
TSP	10	63	61-65	1.6	2.5%
TP	10	2140	2000-2220	76	3.6%
PP	6[a]	129	117-136	7.1	5.5%

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[a] Replicates from a suspension of particles in AAP (-P) algal medium

Table 18. RECOVERY OF ADDED ORTHOPHOSPHATE SPIKES IN DRP AND TSP ANALYSES

A. DRP: Samples filtered before adding spike

Sample No.	DRP[a] In sample	DRP[a] In sample + 25 μg/l spike	Spike Recovery	
	(μgP/l)		(μgP/l)	(% of added P)
56-Niagara R.	37	57	20	80
57-Niagara R.	3	29	26	104
58-Genesee R.	46	68	22	88
59-Oswego R.	57	77	20	80
60-Black R.	11	35	24	96
501-13-Genesee R. Basin	0	25	25	100
		mean values	23	92

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[a] Mean of three replicates

Table 18 (cont'd.). RECOVERY OF ADDED ORTHOPHOSPHATE SPIKES IN DRP AND TSP ANALYSES

B. Total P: Sample B-9 (Unfiltered Madison Urban Runoff)

	TP Concentration	
	In sample	In sample + 500 µgP/l spike
	(µgP/l)	
	1076	1544
	1080	1494
	1028	1540
	1080	1510
	1028	1512
mean values:	$\bar{x}_1$ = 1058	$\bar{x}_2$ = 1520

difference: $(\bar{x}_2 - \bar{x}_1)$ + 462 µgP/l

% of added spike recovered: 462/500 x 100 = 92%

Particulate Inorganic P (PP_i) Extracted by Chemicals

PP_i Extracted by Acid--

Particles were deposited on a membrane filter by passing a known volume of sample through the filter. The particles and the filter were placed into a 50 ml Erlenmeyer flask with 20 ml of acid extraction solution, the filter was scraped with a metal spatula, and the flask was then swirled by hand for 5 to 15 minutes at room temperature. The filter was left in the flask during extraction. The acid extraction solution contained 8.1 ml of concentrated HCl and 1.3 ml of concentrated H_2SO_4, diluted to 2 liters with distilled water (Wentz, 1967). The calculated acid concentration was 0.083N.

The acid extracts were analyzed for DRP after filtration through glass fiber or membrane filters, directly into 25 X 200 mm calibrated test tubes. Prior to 1973, the entire contents of the extraction flask were poured into the filter apparatus, followed by a few small volumes of water used to rinse the flask. This procedure was simplified in later tests, by simply taking a 15 ml aliquot from the extract for filtration. With either procedure, the filtrate was diluted to 50 ml with water and eight ml of color reagent for the analysis of extracted PP_i. Standards and blanks were made with 15 (or 20) ml of extraction solution, to compensate for any pH effects on color development caused by the extraction acids. An average filter blank of 3 μgP/l was calculated from extractions of 20 washed membrane filters, run periodically during 1972 and 1973. This average blank was subtracted from the acid extraction PP_i values.

PP_i Extracted by Anion-Exchange Resin--

Particles isolated from a water sample onto a membrane filter were scraped into 100 ml of distilled water in a 125 ml Erlenmeyer flask or standard BOD (Biochemical Oxygen Demand) bottle; the membrane filter was left in the test flask. One gram of the chloride form was added to the flask, which was then sealed with Parafilm[a] and shaken mechanically for 24 hours at 15 to 22°C on a wrist-action shaker. After the equilibration period, the resin beads were allowed to settle and a 20 ml portion was decanted from the supernatant liquid for filtration and DRP analysis.

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[a] American Can Co., Neenah, Wisc.

The suspension of particulate matter and resin remaining in the test flask was poured through a CRC Micro Sieve[b] (U.S. Series No. 60: 250 microns). Since the resin had been dry-sieved before use (only retained beads were saved for use), and because of resin swelling, almost all of the resin was retained on the wire sieve during the wet-sieving operation, while the particulate matter from the sample was assumed to pass through the sieve quantitatively, leaving only the resin beads on the wire mesh. A few small rinses with water were used to rinse any residual resin out of the test flask onto the sieve.

The slurry of wet resin on the sieve was scraped into a long-stem funnel, which was fitted with a pinch clamp and filled with 1N Na_2SO_4, as shown in Figure 3. The resin was allowed to soak in the funnel for at least 2 hours before the pinch clamp was opened to begin the elution, with fresh 1N $NaSO_4$ added to the reservoir as needed. A 100 ml volume of effluent was collected in a volumetric flask, at an elution rate of approximately 3 ml/minute. The contents of the volumetric flask were mixed well, and a 40 ml aliquot was taken for analysis of resin-bound inorganic P. The aliquot was diluted to 50 ml with water and 8 ml of color reagent for this analysis.

Table 19 summarizes the percent recovery of phosphate spikes added to 1 gram of resin. The average percent recoveries are plotted in Figure 4. This graph was used to find the correction factor necessary for the calculation of resin-bound inorganic P (Appendix A). The inorganic P bound to the resin and the supernatant DRP of the test flask were added to get the apparent "available" P in the flask.

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[b] Chemical Rubber Co., Cleveland, Ohio

Figure 3. Column for solution of phosphate from Dowex 1-X8 resin

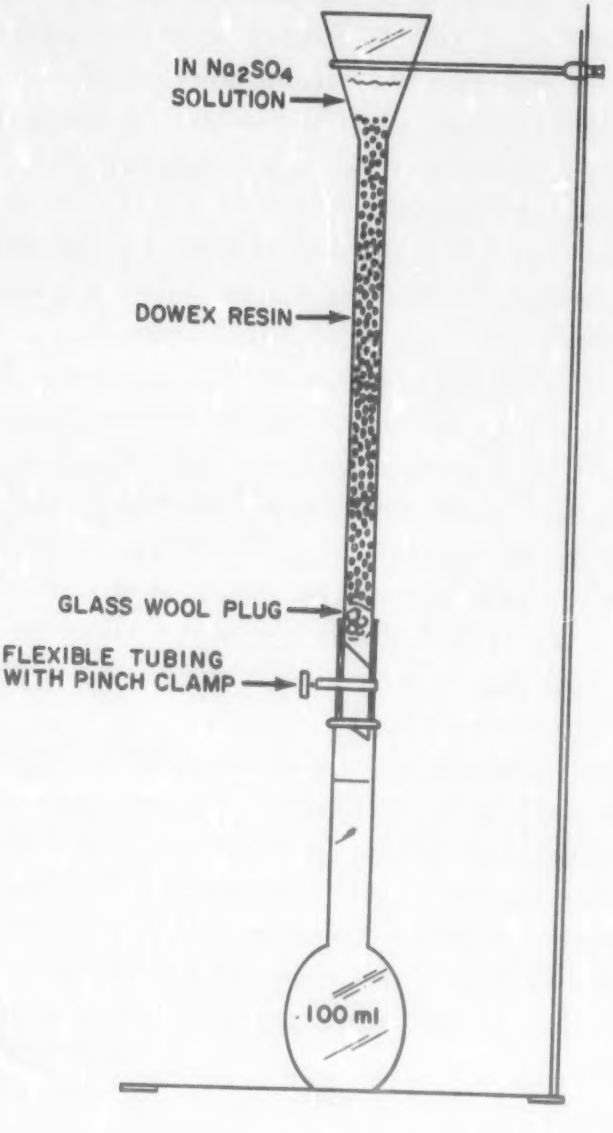
1N Na_2SO_4 SOLUTION
DOWEX RESIN
GLASS WOOL PLUG
FLEXIBLE TUBING WITH PINCH CLAMP
100 ml

Figure 4. Recovery of added phosphate from Dowex 1-X8 resin by 1 N Na_2SO_4 leaching

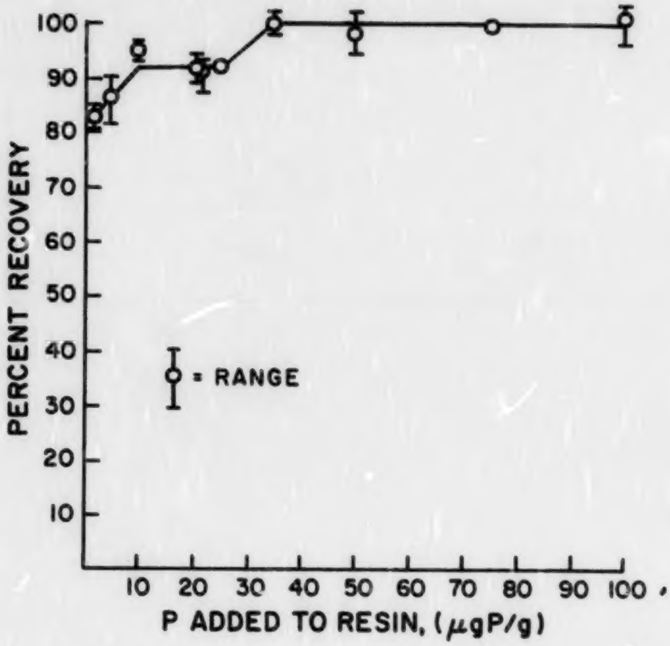

Table 19. RECOVERY OF ADDED ORTHOPHOSPHATE FROM DOWEX 1-X8 ANION-EXCHANGE RESIN BY 1N Na_2SO_4 LEACHING

Ortho P Added to Resin (µgP/g resin)	Number of Determinations	Average % Recovery of Added P	% Recovery Range
2	3	83	80 - 84
5	4	87	81 - 90
10	3	95	93 - 96
21	11	92	89 - 94
22	3	91	87 - 93
25	1	92	92
35	3	100	98 - 102
50	5	98	95 - 102
75	3	99	98 - 99
100	5	101	96 - 103

All standards and reagent blanks were prepared with the same 1N Na_2SO_4 solution used to elute the resin. A resin blank was run with 1 gram of resin in 100 ml of distilled water and was leached along with the samples. The inorganic P in the resin blank eluant was subtracted from that in the sample eluants. The reagent blank was used to correct the standards.

Table 20 summarizes a series of tests which were run to estimate the efficiency of acid, base, and resin extractions. Orthophosphate spikes were added to the extraction solutions prior to introduction of the particulate matter, except in the case of the resin extraction, where the spike was added to a suspension of the particles in water a few minutes before adding the resin. The mean values showed recoveries of 77 to 100 percent for these methods. The low value for the base extraction of Sample No. 34 may have been due to resorption of phosphate in the extraction solution by calcium carbonate (Williams _et_ _al_., 1971b). No such explanation was available for the apparent low recovery from the acid extraction of Sample No. 502-14. The ranges of experimental results with both spiked and unspiked Sample No. 502-14 were unfortunately large relative to the size of the added spike, so that the apparent recovery was not measured precisely. As a conservative estimate of the recovery of acid- or base-extractable P with the methods employed here, about 75 percent was recovered. Generally, all analyses of extractable PP_i and the other P forms discussed above were performed in triplicate.

ALGAL ASSAYS OF PARTICULATE PHOSPHORUS

Direct estimation of the available phosphorus in natural particulate matter was made by growing _Selenastrum capricornutum_ in phosphorus-free medium containing the particulate matter. The procedure consisted of four steps:

Table 20. RECOVERY OF ORTHOPHOSPHATE SPIKES ADDED TO ACID, BASE, AND RESIN EXTRACTION SOLUTIONS BEFORE EQUILIBRATION WITH SAMPLE PARTICLES

A. 0.083N Acid Extractions

1.) Sample No. 42 (Genesee R.) Particles

DRP Concentrations in Extracts after Equilibration with Particles	
Unspiked extraction solution	Spiked extraction solution (+200 µgP/l)
(µgP/l)	
279	480
292	504
308	496

mean values $\bar{x}_1 = 293$ $\bar{x}_2 = 493$

difference: $(\bar{x}_2 - x_1) = 200$ µgP/l

% of added spike recovered: 200/200 x 100 = 100%

2.) Sample No. 502-14 (Genesee R. Basin Sample) Particles

DRP Concentrations in Extracts after Equilibration with Particles	
Unspiked extraction solution	Spiked extraction solution (+100 µgP/l)
(µgP/l)	
311	381
332	409
337	421

mean values $\bar{x}_1 = 327$ $\bar{x}_2 = 404$

difference: $(\bar{x}_2 - x_1) = 77$ µgP/l

% of added spike recovered: 77/100 x 100 = 77%

Table 20 (cont'd). RECOVERY OF ORTHOPHOSPHATE SPIKES ADDED TO ACID, BASE, AND RESIN EXTRACTION SOLUTIONS BEFORE EQUILIBRATION WITH SAMPLE PARTICLES

B. 0.1N NaOH Extraction of Sample No. 34 (Genesee R.) Particles

	DRP Concentration in Extracts after Equilibration with Particles	
	Unspiked extraction solution	Spiked extraction solution (+238 μgP/l)
	46	224
	58	248
	50	237
mean values	$\bar{x}_1$ = 51	$\bar{x}_2$ = 236

difference: $(\bar{x}_2 - \bar{x}_1)$ = 185 μgP/l

% of added spike recovered: 185/238 x 100 = 78%

C. Anion-Exchange Resin Extraction of Sample No. 507-14 (Genesee R. Basin Sample) Particles

	Total DRP Concentration in Supernatant and in Na_2SO_4 Extract of Resin after Equilibration of Resin and Particles	
	Unspiked extraction solution (H_2O + resin)	Spiked extraction solution (H_2O+100 μgP/l = resin)
	(μgP/l)	
	27	126
	30	128
	28	127
mean values	$\bar{x}_1$ = 28	$\bar{x}_2$ = 127

difference: $(\bar{x}_2 - x_1)$ = 99 μgP/l

% of added spike recovered: 99/100 x 100 = 99%

1.) preparation of the inoculum, 2.) preparation of the test flasks, 3.) growth of the algae, and 4.) quantitation of the algal growth. Steps No. 1 and No. 3 were general procedures which were also used for soluble P bioassays (discussed below). Steps No. 2 and No. 4 were performed differently in assays of particulate P than in assays of soluble P.

Preparation of the Algal Inoculum

A unialgal culture of *Selenastrum capricornutum*, initially obtained from Dr. G. P. Fitzgerald[a], was subcultured at approximately biweekly intervals by transferring a small volume of mature cell culture to a liter of freshly prepared, sterile growth medium. The medium used for the stock culture transfers was an enriched Algal Assay Procedure (AAP; EPA, 1971) medium containing three times the nitrate and phosphate levels of AAP medium.

The standard AAP medium composition is given in Table 21. Six stock solutions of the individual macronutrient salts were made with reagent grade chemicals dissolved in glass distilled water at 1000 times the final concentration in AAP medium. A single stock micronutrient solution was made by combining all of the micronutrient salts in glass distilled water at 1000 times their final concentrations in the AAP medium. The complete medium was made by combining 1 ml volumes of all stock solutions and diluting to 1 liter with glass distilled water.

The algal inoculum for growth assays was washed from its stock culture medium by an alternating sequence of three centrifugations (1500 rpm for 20 min.) and three resuspensions of the pelletized cells in about 10 ml of fresh AAP medium, minus phosphate (AAP(-P)). Following the third

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[a] Water Chemistry Laboratory, University of Wisconsin, Madison

Table 21. COMPOSITION OF STANDARD AAP ALGAL MEDIUM

A. Macronutrients

Compound	Concentration (mg/l)	Element	Concentration (mg/l)
$NaNO_3$	25.500	N	4.200
K_2HPO_4	1.044	P	0.186
$MgCl_2$	5.700	Mg	2.904
$MgSO_4 \cdot 7H_2O$	14.700	S	1.911
$CaCl_2 \cdot 2H_2O$	4.410	C	2.143
$NaHCO_3$	15.000	Ca	1.202
		Na	11.001
		K	0.469

B. Micronutrients

Compound	Concentration (µg/l)	Element	Concentration (µg/l)
H_3BO_3	185.520	B	32.460
$MnCl_2$	264.264	Mn	115.374
$ZnCl_2$	32.709	Zn	15.691
$CoCl_2$	0.780	Co	0.354
$CuCl_2$	0.009	Cu	0.004
$Na_2MoO_4 \cdot 2H_2O$	7.260	Mo	2.878
$FeCl_3$	96.000	Fe	33.051
$Na_2EDTA \cdot 2H_2O$	300.000		

(Source: EPA, 1971)

resuspension, the cell suspension was counted on a hemocytometer under a microscope, to determine the dilution needed to achieve the proper volume of suspension and cell concentration in the suspension. One ml of a 27 X 10^4 cells/ml inoculum cell suspension was required for each assay test flask. Except in the transfer of stock cultures, sterile techniques were not used in the handling of the algal cells or the preparation of algal assay media.

Preparation of the Test Flasks

All assay cultures were grown in 50 ml Erlenmeyer flasks cleaned with hot, concentrated nitric acid and rinsed with regular distilled water (six times), followed by glass distilled water (four times).

Sample particles isolated on membrane filters, as described above, were scraped into AAP(-P) medium, and the suspension was shaken to distribute the particles uniformly in the medium. Five 25 ml aliquots were taken with a 25 ml graduated cylinder and poured into the culture flasks. The remainder of the suspension was saved for PP analysis. One ml of glass distilled water and 1 ml of inoculum cell suspension were added to each flask to give a final culture volume of 27 ml, with an initial algal cell count of 1 X 10^4 cells/ml. Comparison tests were also run with autoclaved particulate matter in AAP(-P) medium. The suspensions were autoclaved for 15 minutes at 15 psi, cooled, and bubbled with carbon dioxide, if necessary, to adjust the pH to 7-8 before use in a bioassay.

Standard culture flasks contained 25 ml of AAP(-P) medium plus 1 ml of inoculum cell suspension and 1 ml of standard KH_2PO_4 solution (made with glass distilled water), which was prepared from the same primary standard solution used for chemical analyses. Since the volume of the standard cultures, like the sample cultures, was 27 ml, the standard KH_2PO_4 solutions contained 27 times their final concentrations in the assay flasks. Blanks were prepared in the same manner as the standards, except that 1 ml of water was substituted for 1 ml of standard P solution. Generally, five replicate flasks were run for blanks, standards and samples. All flasks were stoppered with cotton plugs.

Growth of the Algae

The culture flasks were incubated under approximately 400 foot-candles of continuous light provided by cool white fluorescent lamps in a constant temperature room set at 20°C. Under the lights, the temperature was 22±2°C. The flasks were swirled daily by hand during their incubation.

Tests were run to determine the proper incubation period for the bioassays with Selenastrum. Figure 5 shows the results of one of the tests, where the average light absorbance (750 nm) of five standard flasks at each phosphorus level was followed versus time of incubation. The average absorbance values at all levels of P appeared to have leveled off between 15 and 18 days. After 18 days, some of the cultures showed gradual increases in absorbance. Based on such data, an 18 day incubation period was chosen for the growth assays. Available phosphorus from particulate matter was thus operationally defined by the population of algae which the particulate phosphorus could produce in a phosphorus-free medium in 18 days, compared to the growth from orthophosphate in the same medium. Figure 6 shows the relationship between algal growth after 18 days and the initial concentration of orthophosphate in the cultures. Up to 200 μgP/l, phosphorus appeared to be clearly limiting the algal growth reached in 18 days. Above 200 μgP/l, however, the slope of the curve changed but did not appear to reach the well-defined plateau (slope=0) expected if phosphorus

Figure 5. Absorbance of *Selenastrum* cultures in AAP medium vs. time

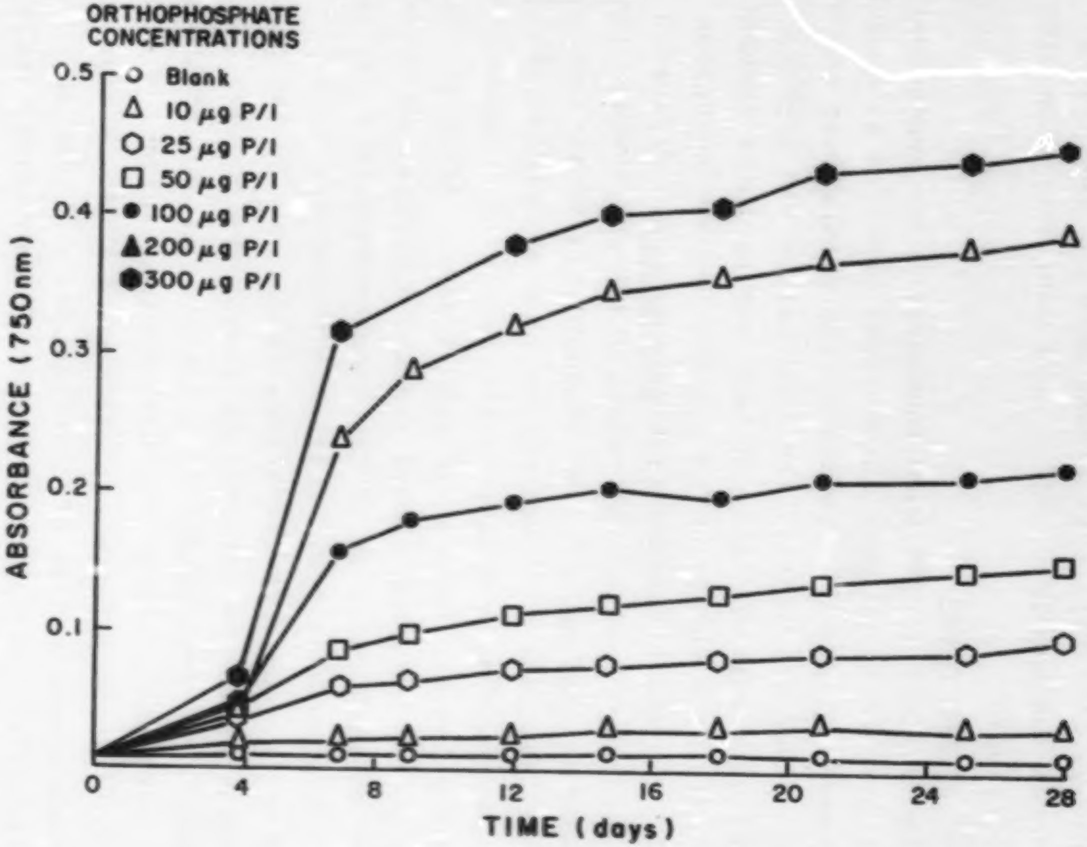
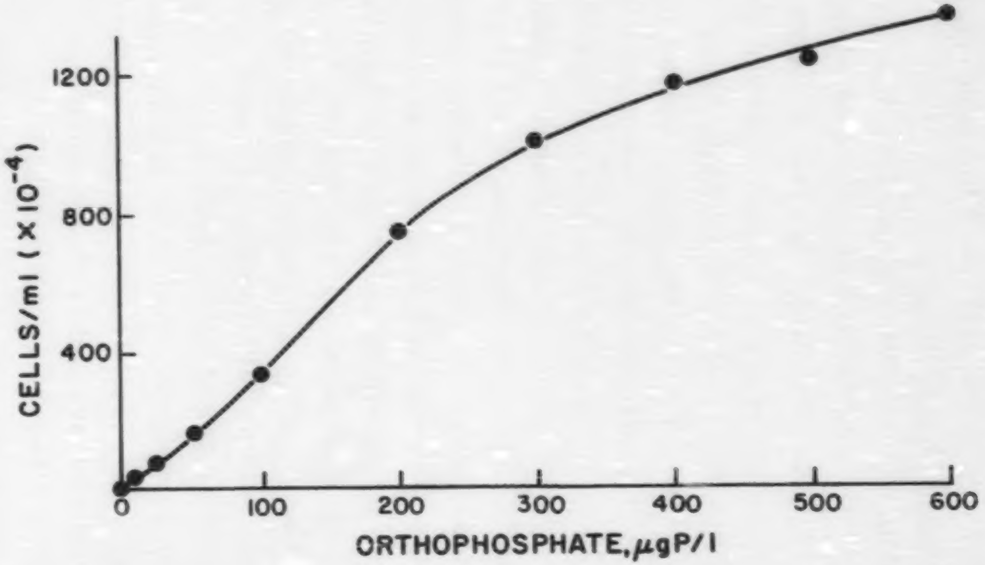


Figure 6. Standard curve for cell counts of S. capicornutum after 18 days vs. orthophosphate concentration (0-600 μgP/l)



were no longer limiting the growth. A possible explanation may be that phosphorus was still nutritionally limiting, but self-shading at high culture densities restricted the light intensity for part of the culture in each flask, causing a partial growth limitation and the rounding off of the curve in Figure 6 . However, to avoid such effects, attempts were made to work in the range of 0 to 200 μgP/l.

Quantitation of Algal Growth

Following the incubation period, the culture flasks were shaken and, if necessary, scraped with a spatula to loosen cells from the flask walls. A small volume was withdrawn and placed into a hemocytometer counting chamber under a microscope. The Selenastrum cells in the chamber were quantitated by counting 10 fields of at least 50 cells per field. An exception to this procedure was made for the blanks or 10 μgP/l standards, where 10 fields of 10^{-4}ml volume were counted. A second exception was made for bioassays of unfiltered New York precipitation samples in which very little algal growth was apparent. These assay cultures were compared to standards with 10 μgP/l by measurement of light absorbance at 750 nm wavelength (A_{750}). Since the samples contained particulate matter which added to the absorbance from algae, the apparent growth in the cultures was biased positively. If such positively biased A_{750} values were found to be less than the A_{750} values for the 10 μgP/l standards, then the actual growth in the samples must have been less than the growth in the 10 μgP/l cultures, and the available P concentration in the samples was reported as less than 10 μgP/l.

The 10 hemocytometer field counts from each sample flask were averaged, and the mean field count was compared to a standard curve like that in Figure 6 to find the apparent available P in the flask. Standard curves were drawn through points which represented the average cell count from five replicate flasks, where each flask cell count was the average of ten hemocytometer field counts. Thus, each point was derived from 50 field counts from each phosphorus level. As shown in Appendix A , a "smallest detectable" phosphorus concentration was calculated from the mean and standard deviation of the blank flasks. Sample flasks with cell counts lower than a specified cutoff value ("C" in Appendix A) were assigned an apparent P value of less than the smallest detectable P concentration, although the lack of growth may have been due to an inhibition unrelated to the presence or absence of phosphorus.

Table 22 shows the recovery of orthophosphate spikes (50 μgP/1) added to two suspensions of river water particles which had been autoclaved before assay in AAP(-P) medium. Recoveries of only 76 to 86 percent were found for the two samples, which had PP concentrations of 79 to 115 μgP/1, respectively, before autoclaving. The autoclaving procedure may have solubilized some of this particulate phosphorus, but evidently the PP remaining sorbed some of the added orthophosphate spike, and rendered it unavailable to Selenastrum. The different recoveries for the two samples may have been related to the different origins of the particulate matter, namely the Niagara and Genesee Rivers. As a comparison test, 25 μgP/1 spikes were added to an autoclaved, filtered Niagara River sample which had been supplemented with AAP (-P) medium, and whose growth was quantitated by cell counts, as in the particulate P bioassays. The spikes were quantitatively recovered in this test, as there was no particulate matter in the test flasks.

Table 22. RECOVERY OF ORTHOPHOSPHATE SPIKES, ADDED TO AAP(-P) MEDIUM CONTAINING PARTICULATE MATTER, IN SELENASTRUM GROWTH BIOASSAYS

Sample No.	PP conc. in cultures (µgP/l)	Apparent Available P[a] In sample (µgP/l)	Apparent Available P[a] In sample +50 µgP/l spike (µgP/l)
No. 50- autoclaved Niagara R. particles	79	46	--
		43	88
		46	78
		51	82
		41	85
mean values		$\bar{x}_1$ = 45	$\bar{x}_2$ = 83

difference: $(\bar{x}_2 - \bar{x}_1)$ = 38 µgP/l

% of added P recovered: 38/50 x 100 = 76%

Sample No.	PP conc. in cultures (µgP/l)	Apparent Available P[a] In sample (µgP/l)	Apparent Available P[a] In sample +50 µgP/l spike (µgP/l)
No. 51- autoclaved Genesee R. particles	115	52	--
		51	--
		45	91
		43	93
		44	85
mean values		$\bar{x}_1$ = 47	$\bar{x}_2$ = 90

difference: $(\bar{x}_2 - \bar{x}_1)$ = 43 µgP/l

% of added P recovered: 43/50 x 100 = 86%

Comparison Test: Sample No. 56 (Niagara R.), Autoclaved, Filtered; and Supplemented with AAP(-P) Nutrients before Assay

	Apparent Available P[a] In Sample (µgP/l)	Apparent Available P[a] In sample + 25 µgP/l spike (µgP/l)
	7	31
	7	34
	8	30
	8	34
	5	32
mean values	$\bar{x}_1$ = 7	$\bar{x}_2$ = 32

difference: $(\bar{x}_2 - \bar{x}_1)$ = 25 µgP/l

% of added P recovered: 25/25 x 100 = 100%

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[a] All test cultures were quantitated by comparing cell counts of sample cultures to standard curves of cell count vs. P concentration in AAP standard cultures.

ALGAL ASSAYS OF SOLUBLE PHOSPHORUS

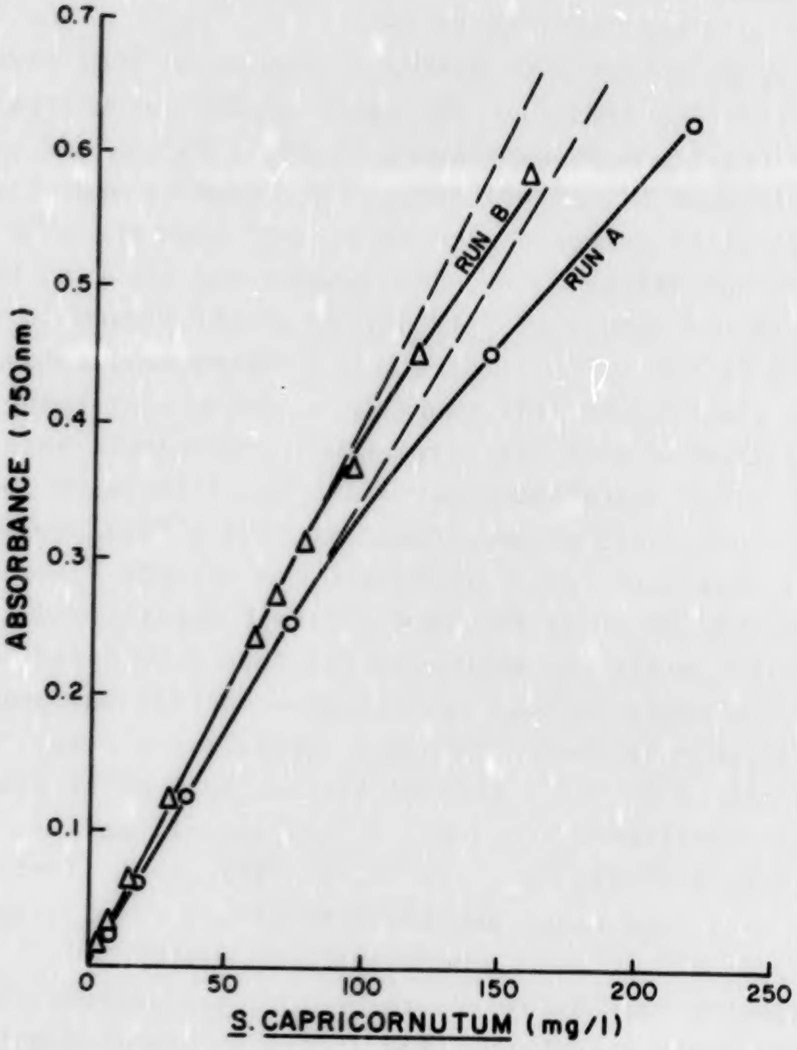
For estimation of the potentially available fraction of TP in river waters, unfiltered samples were first autoclaved for 15 minutes at 15 psi, then were cooled and filtered through 0.45 micron pore size membrane filters. If necessary, carbon dioxide was bubbled through the filtrates to bring their pH to 7-8.

Twenty ml aliquots of filtrate from autoclaved river water, containing less than 200 μgP/l of TSP, were pipetted into five replicate 50 ml culture flasks. Five ml of freshly prepared 5X AAP(-P) medium was added to each flask to give 25 ml of solution with 1X AAP(-P) plus the nutrients present in the sample water. Phosphorus was shown to be limiting to the growth of Selenastrum in AAP medium in the range of 0 to 200 μgP/l (Figure 6). Therefore, phosphorus had to be limiting in this range in a medium containing AAP(-P) nutrients plus the nutrients in the sample water. One ml of glass distilled water and 1 ml of inoculum cell suspension was added to each flask to give a final volume of 27 ml. Standard and blank flasks for soluble P assays were identical to those for particulate P assays, and the same culture conditions were used for both procedures.

For the assay of soluble P in urban runoff samples, two dilutions were used. Twenty ml of filtered runoff was enriched with 5 ml of 5X AAP(-P) medium, or 5 ml of filtered runoff was enriched with 20 ml of 5/4X AAP(-P) medium. In either case, 1 ml of glass distilled water and 1 ml of inoculum cell suspension was added to give a final volume of 27 ml.

Following the 18-day growth period, the cultures were shaken and their absorbance (A), at 750 nm wavelength was measured in a 1-inch Spectronic 20 cell. Figure 7 demonstrates the optical properties of this quantitation

Figure 7. Absorbance vs. dry weight of *S. capricornutum*



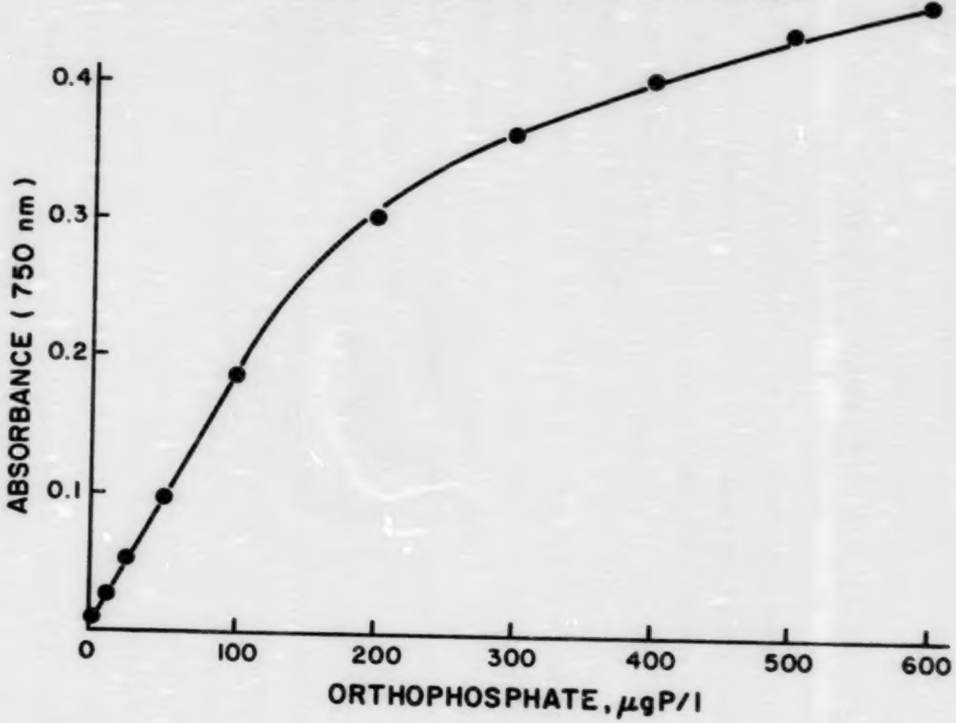
method. Two different stock Selenastrum cultures were quantitated by dry weight measurements, and various dilutions and concentrations of the cultures were quantitated by A_{750} measurement. The A_{750} values were plotted versus dry weight values computed from the dry weight concentrations of the two stock cultures. There was a linear relationship (dashed lines in Fig. 7) between dry weight and absorbance in the two tests shown, up to about $A = 0.350$. At higher absorbance values, there was a negative deviation from linearity, as the absorbance per unit of algae decreased.

As demonstrated by the standard curve of A_{750} (18-day old cultures) versus phosphorus concentration in Figure 8, the apparent final growth levels for high values of P did not follow the same linear relationship as did the growth at lower levels of P. The observed negative deviation from the initial linear function may have been partially due to the optics of culture measurement in the Spectronic 20 (see Figure 7) , where dense cultures show less absorbance per unit weight of algae than do dilute cultures. In addition, some physical growth factor such as light may have limited the growth at high population densities, where self-shading of cells becomes important. The absence of a well-defined plateau above 200 μgP/l indicates that P may still have been a limiting nutrient above that P level.

Each standard curve was constructed with average A_{750} values from five replicate AAP standard cultures at each P level. Minimum detectable values of available P were calculated as shown in Appendix A.

In the bioassays of particulate matter in AAP(-P) medium, an assumption was made that the particles acted only as sources or sinks of available P and did not

Figure 8. Standard curve for absorbance of S. capricornutum after 18 days vs. orthophosphate concentration (0-600 μgP/l)



significantly alter the composition of the algal growth medium, so that comparisons between particulate suspension in AAP (-P) medium and standard AAP medium cultures containing orthophosphate could be made. In the case of the bioassay of filtered natural water samples, however, supplementation of the filtrates with AAP (-P) nutrients created a new medium, containing both AAP (-P) nutrients and the nutrients originally present in the filtrate. Although both the supplemented sample medium and the AAP standard medium may have been limited by phosphorus, their response curves (growth as indicated by A_{750} vs P) may not have been the same shape, as illustrated in Figure 9 . In the hypothetical case shown in the figure, comparison of the sample culture growth to the AAP standard curve (dotted lines) would result in an underestimation of the available P in the sample.

An actual partial sample response curve was drawn by adding orthophosphate spikes to Sample No. 52 (autoclaved, filtered, and supplemented with AAP (-P)), as shown in Figure 10 . Since the phosphorus level in the sample was fairly high, no information about the lower portion of the response curve was given by this experiment. However, the upper side of the curve was different from the upper portion of the AAP standard curve, so the curves were probably not alike at lower values of P. The unspiked sample showed an apparent available P value of 76 μgP/l when its average A_{750} value was compared to the AAP standard curve (dotted lines). This value was an overestimate, since the TSP in the culture flasks was measured as 54 μgP/l before the bioassay. Likewise, the sample with a 25 μgP/l spike showed a P value of 51 μgP/l above the unspiked sample, an overestimate of the spike by 26 μgP/l.

Figure 9. Hypothetical case for underestimation of available P in a filtered sample bioassay.

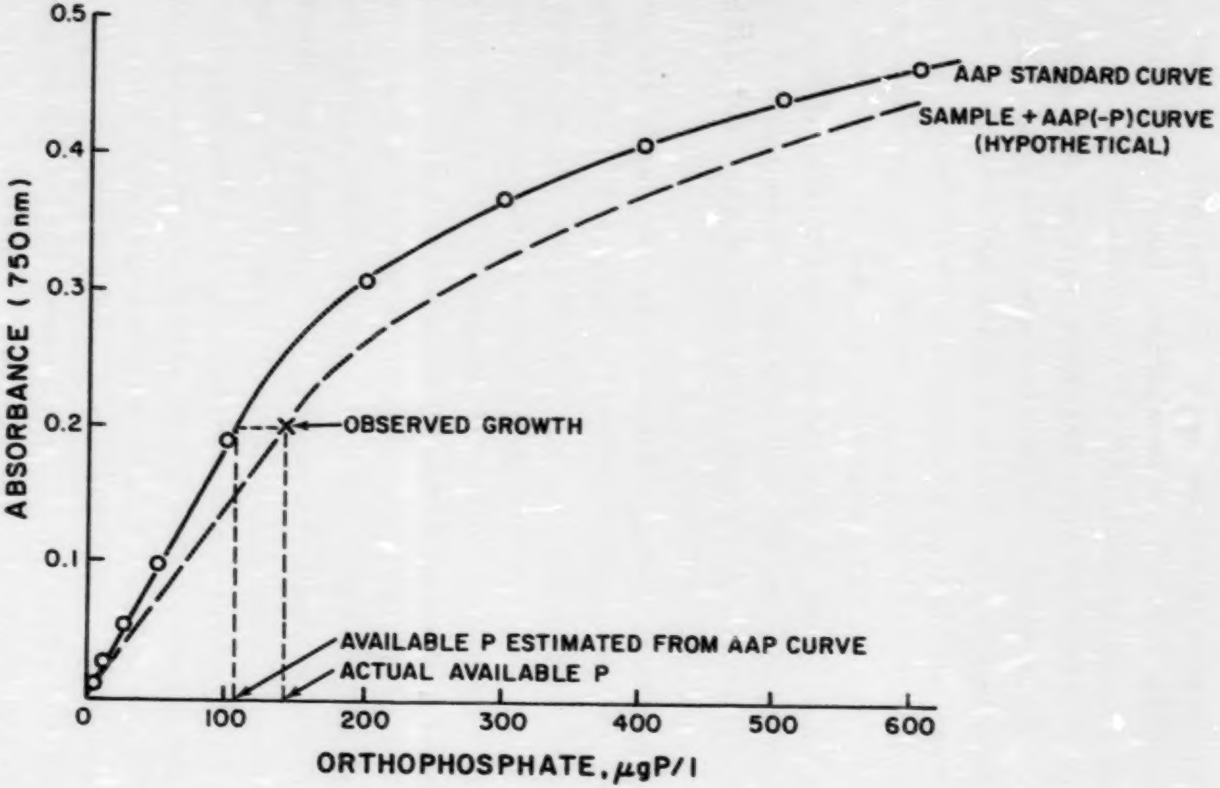
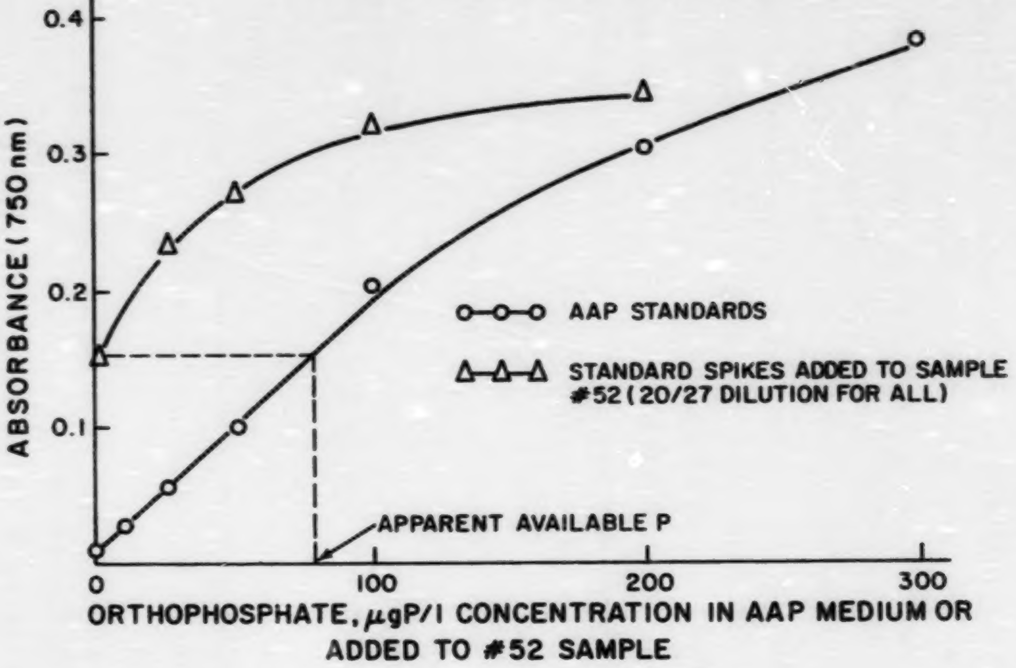


Figure 10. Standard curves for absorbance of S. capricornutum vs. orthophosphate concentration in AAP medium or in spikes added to sample #52 (Oswego River) water (Autoclaved, Filtered)



In order to determine whether the optical properties of 18-day old Selenastrum cultures grown in sample water supplemented with AAP(-P) and in AAP medium were different, correlation curves relating A_{750} and cell counts were drawn. Figure 11 shows the correlation found for AAP standard cultures. A least-squares slope of 5.6×10^{-8} absorbance units/cell count unit was obtained, with a correlation coefficient of $r=0.92$. In contrast, a series of Niagara, Genesee, and Oswego River samples showed a slope of 8.0×10^{-8} absorbance units per cell count unit, with a correlation coefficient of $r=0.98$ (Figure 12).

The ratio of the sample to standard slopes ($8.0/5.6=1.43$) indicated that for a given cell count, the net A_{750} for a sample culture would be about 1.43 times the net A_{750} from an AAP standard culture with the same final cell count. When the net mean A_{750} value for Sample No. 52 (Figure 6.8) was divided by the slope ratio of 1.43, a corrected net mean A_{750} value was obtained.

Mean observed A_{750}, sample No. 52 = 0.151
Mean blank culture A_{750} = 0.010
Net mean A_{750}, sample No. 52 = 0.151-0.010 = 0.141
Corrected net mean A_{750}, sample No. 52 = 0.141/1.43
= 0.099

Addition of the blank A_{750} value to the corrected sample A_{750} value gave an absorbance value which was then compared to an AAP standard curve of A_{750} versus P concentration:

Corrected net mean A_{750} + blank A_{750} = 0.099 + 0.010
= 0.109 (Standard curve P value for 0.109 was 53 μgP/l)

The resulting P value was in agreement with the TSP level of 54 μgP/l in the culture flask prior to assay. Unfortunately, the DRP level in the culture flasks was not measured.

Figure 11. Correlation between absorbance and cell counts for *S. capricornutum* in AAP medium

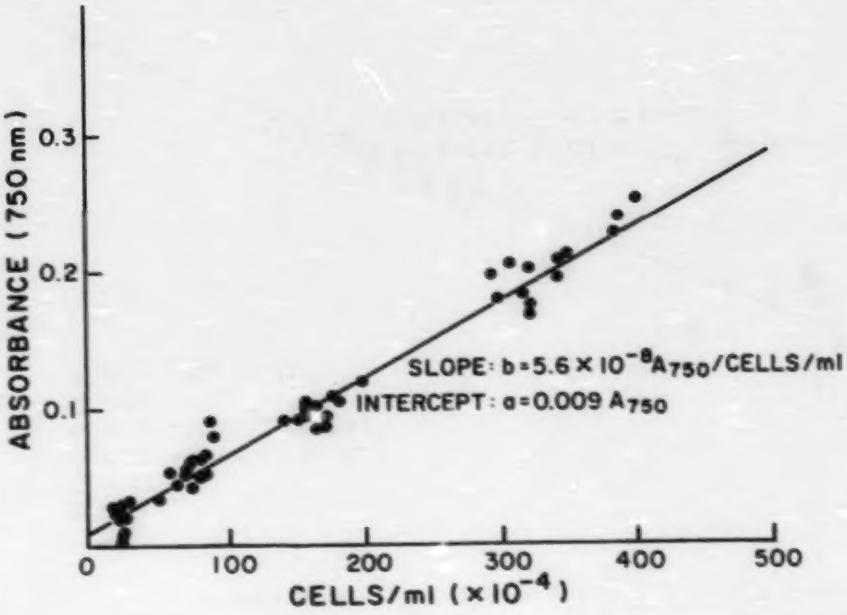
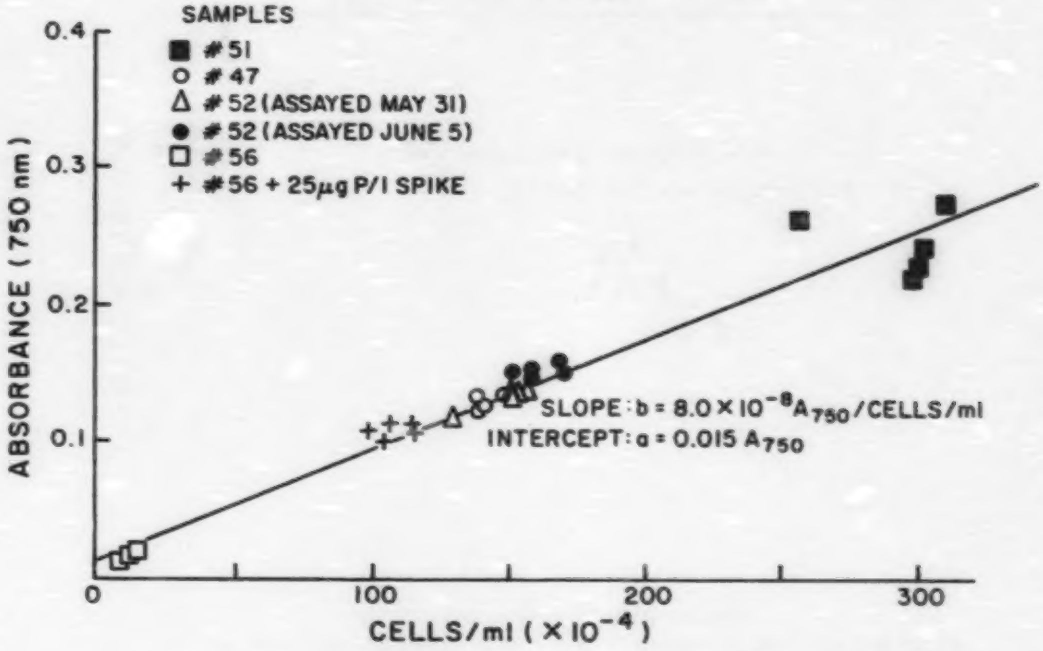


Figure 12. Absorbance vs. cell counts for S. capricornutum grown in autoclaved, filtered river waters supplemented with 1x AAP-P medium



The preceding method of calculation essentially transformed the growth response of sample cultures to a cell count basis of quantitation, with the assumption that a unit of P in a sample culture produced the same concentration of cells as a unit of P in a standard AAP culture. This assumption was tested by counting assay Samples No. 56 and No. 56 + 25 μgP/l spike with a hemocytometer (see Table 22); complete recovery of the spike was found in this test. Table 23 shows a comparison between TSP and bioassay results as measured by uncorrected A_{750} data or by hemocytometer cell counts. Bioassay results above TSP were considered overestimates of P availability, since TSP represented the theoretical maximum value of available P. In all the tests shown in Table 23 except the very dilute Niagara River Sample No. 56, the uncorrected A_{750} data overestimated available P. Direct cell counting, in contrast, overestimated available P in only one of the samples (No. 51). Standard AAP calibration curves of cell count versus P concentration were used to quantitate those (autoclaved, filtered) samples which were counted on a hemocytometer.

As a further test of the cell count basis for bioassay calculations, the A_{750} data from spikes added to Sample No. 52 (Figure 10) were corrected as described above. The results are presented in Table 24. Recoveries of 116 and 92 percent were found for 25 and 50 μgP/l spikes, respectively, while higher spike levels were recovered less completely. The low recoveries at high spike levels was probably due to growth limitation by other factors. For other samples, the use of corrected absorbance values yielded P recoveries about 12 percent or less from the expected recovery (Table 25).

Table 23. COMPARISON OF TSP IN CULTURE FLASKS AND BIOASSAY RESULTS, AS COMPUTED FROM DIRECT CELL COUNTS AND FROM UNCORRECTED A_{750} DATA

Sample[d]	TSP in Culture Flask	Mean Apparent Available P from: A_{750}[a] data	Mean Apparent Available P from: Cell counts[b]
	(µgP/1)		
No. 56 (Niagara R.)	13	7	7
No. 51 (Genesee R.)	83	139	92
No. 47 (Oswego R.)	53	61	41
No. 52 (Oswego R., Assayed May 31)	54	69	50
No. 52 (Oswego R.; Assayed June 5)	54	76	50

[a] Uncorrected A_{750} data was compared to AAP standard curves of A_{750} vs. P concentration to find apparent available P.

[b] Sample cell counts were compared to AAP standard curves of cell count vs. P concentration to find apparent available P.

[d] All samples were autoclaved, filtered, and supplemented with AAP(-P) nutrients before assay.

Table 24. RECOVERY OF ORTHOPHOSPHATE SPIKES ADDED TO AUTOCLAVED, FILTERED OSWEGO R. SAMPLE NO. 52, AS CALCULATED USING CORRECTED NET A_{750} BIOASSAY DATA

Sample	Net mean A_{750} measured[a]	Corrected net mean A_{750} + blank A_{750}[b]	Apparent Available P	Spike Recovery	
			(μgP/1)	(μgP/1)	(% of added P)
No. 52	0.141	0.109	53	--	--
No. 52 + 25 μgP/1	0.217	0.162	82	29	116
No. 52 + 50 μgP/1	0.259	0.191	99	46	92
No. 52 + 100 μgP/1	0.309	0.226	122	69	69
No. 52 + 200 μgP/1	0.331	0.241	134	81	40

[a] Mean A_{750} measured minus the A_{750} average of the blank cultures

[b] Net mean A_{750} divided by 1.43

Table 25. RECOVERY OF ORTHOPHOSPHATE SPIKES ADDED TO AUTOCLAVED, FILTERED RIVER WATERS, AS CALCULATED USING CORRECTED NET A_{750} BIOASSAY DATA

Sample	Net mean A_{750} measured[a]	Corrected[b] net mean A_{750} + blank A_{750}	Apparent Available P (μgP/l)	Spike recovery (μgP/l)
No. 56 (Niagara R.)	0.015	0.014	5	--
No. 56 + 25 μgP/l	0.103	0.076	32	27
No. 57 (Niagara R.)	0.007	0.009	3	--
No. 57 + 25 μgP/l	0.098	0.073	30	27
No. 58 (Genesee R.)	0.136	0.100	42	--
No. 58 + 25 μgP/l	0.222	0.160	70	28
No. 59 (Oswego R.)	0.154	0.122	52	--
No. 59 + 25 μgP/l	0.238	0.172	76	24
No. 59 (½X)[c]	0.075	0.057	24	--
No. 59 (½X)[c] + 25 μgP/l	0.181	0.131	57	33
			Average P recovery	28 μgP/l
			P added	25 μgP/l
			Average % of added P recovered	112%

---

[a] Mean A_{750} measured minus the A_{750} average of the blank cultures

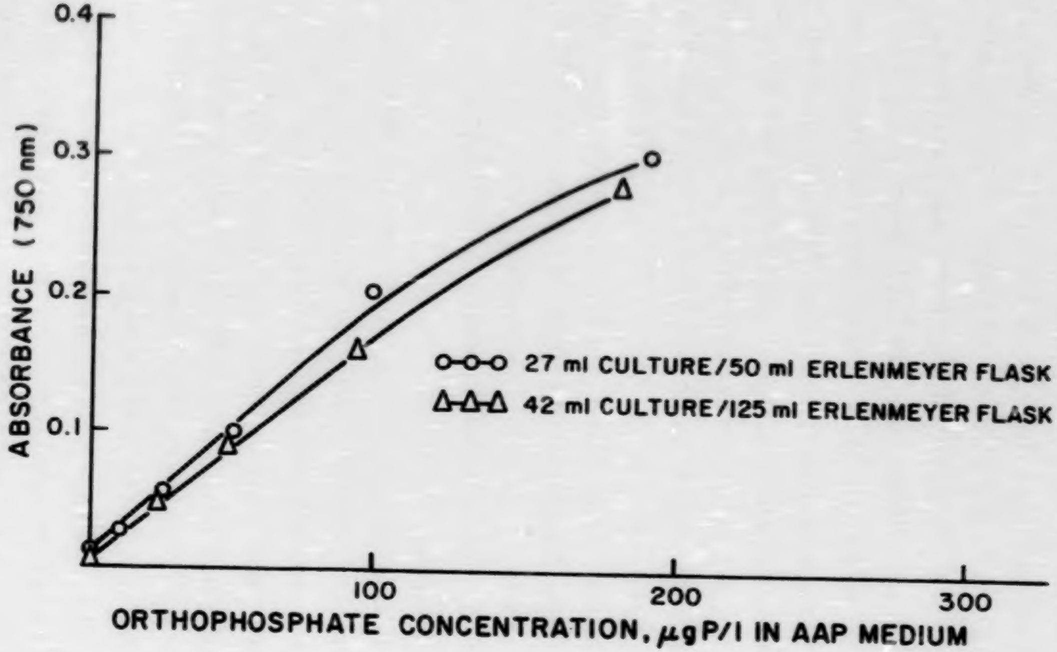
[b] Net mean A_{750} divided by 1.43

[c] Autoclaved, filtered sample No. 59 was diluted 1 + 1 with H_2O before the bioassay

In summary, the results of bioassays with autoclaved river waters were quantitated in two ways: 1.) with uncorrected A_{750} data, and 2.) with absorbance data corrected to a cell count basis. The correlation curve in Figure 12 was made with only a few samples from the Genesee (No. 51), Niagara (No. 56 and No. 56 + 25 μgP/l spike), and the Oswego (No. 47 and No. 52) Rivers. Consequently, there is not extensive data to show that the ratio of 1.43, between sample and standard culture net mean A_{750} values holds for all of the samples from these sources which were bioassayed. Nor is there any evidence that the autoclaved Black River or filtered Madison urban runoff samples would show the same ratio, since no samples from these sources were included in the regression analyses outlined above. Consequently, none of the Black River or filtered Madison runoff A_{750} bioassay data were corrected with the ratio method before comparison to standard AAP curves of A_{750} vs. P concentration. In all bioassays, the criteria given in Appendix A were used to reject outliers among the replicate culture flasks.

As a check to insure that the culture volume and flask size were not restrictive to algal growth due to a low surface to volume ratio, a comparison growth test was made with a surface volume ratio suggested by the EPA (1971). The 27 ml culture volume/50 ml flask ratio used in the studies reported here was compared to the 42 ml culture volume/125 ml flask ratio suggested for standard AAP tests (EPA, 1971). Figure 13 shows that the results of the two procedures were similar. As noted above, the flasks were swirled daily to provide exchange of gases between the culture and the atmosphere.

Figure 13. Standard curves for S. capricornutum cultured in 50 ml and 125 ml flasks



ALGAL ASSAYS OF UNFILTERED RAIN GAGE SAMPLES

The combined available phosphorus from soluble and particulate forms in a rain sample was estimated by growing *Selenastrum* in an unfiltered sample. Twenty ml of sample or an aliquot of sample diluted to 20 ml was enriched with 5 ml of 5X AAP(-P) medium, and 1 ml of water plus 1 ml of inoculum cell suspension was added to give a final volume of 27 ml. After the 18 day growth period, the *Selenastrum* was counted on a hemocytometer, and quantitated as in the assays of particulate phosphorus.

DARK INCUBATIONS OF UNFILTERED SAMPLES

Samples of unfiltered water were stored at 15 to 22°C in darkness for up to 50 days, in order to follow the changes in DRP as an indicator of net inorganic phosphorus release or immobilization in the samples. Three types of dark incubation were compared: 1.) sample alone, 2.) sample plus anion-exchange resin, and 3.) sample plus chloroform. The general procedures used for these tests are described in this section.

Samples Incubated Alone

Three acid-washed (1:1 conc. HCl:water by volume) 125 ml Erlenmeyer flasks or standard BOD bottles were rinsed with sample and allowed to drain. One hundred ml of well-mixed sample was then measured with a graduated cylinder and poured into each flask. The flasks were plugged with cotton and covered with a black plastic shroud for storage in the algal culture room on an unlighted shelf. Each test flask was swirled daily by hand to mix the samples and provide adequate aeration. After approximately 13, 25, and 50 days of storage, the flasks were sampled by filtering a well-mixed portion through a glass fiber or membrane filter. The filtrate (20 ml) was collected in a calibrated 25 X 200 mm test tube, for subsequent DRP analysis. The remaining sample in the test flask was saved for further incubation if necessary.

Samples Incubated with Resin

Six flasks containing 100 ml of sample were given 1 gram of Dowex 1X8 resin per flask. Three flasks with 1 gram of resin in 100 ml of water were set up as resin blanks. The resin was air-dried and dry-sieved as described above for resin extraction of PP, before use in the incubations. On each scheduled sampling date, two of the flasks with sample plus resin and one of the resin blank flasks were analyzed by the same procedure used in the extraction of PP by resin.

Two variations of the general sample ± resin test were attempted. In the first variation, river water (300 ml) was mixed with 300 ml of Lake Ontario water collected near the mouth of the river. Bottles with this mixture were incubated for 100 days, with and without resin. Bottles with lake water alone and with resin were carried along as controls, so that the available P from the river water could be calculated. On the sampling dates, the bottles without resin were sampled as described above for "sample alone." The contents of the bottles with resin were poured through a No. 60 sieve and placed in a large funnel to catch the liquid which passed through the sieve for further incubation. The resin on the sieve was extracted with 1N Na_2SO_4 as outlined above. After taking an aliquot from the liquid which had passed through the sieve (for supernatant DRP analysis), 1 gram of fresh resin was placed in the remaining liquid for the next period of incubation. A volumetric correction factor was applied to each resin-bound DRP value to account for the volume of sample which was extracted by the resin. The cumulative resin-bound DRP at each point during the incubation was added to the prevailing supernatant DRP level at that point in time, to find the cumulative "available" P as a function of time.

In the second variation of the general procedure, particles isolated on membrane filters were scraped into 100 ml of water taken from the lake which was expected to receive the runoff or river water carrying the particles. Three flasks without resin and six flasks with resin were incubated, for the suspensions of particles and for the lake water controls. In all other respects, the procedure for this test was the same as that used for unfiltered samples ± resin.

Samples Incubated with Chloroform

One ml of reagent grade chloroform ($CHCl_3$) was added to 100 ml of unfiltered sample of river water in an acid-washed test flask. The concentration of chloroform was in excess of the saturation concentration, as evidenced by a bubble of chloroform in the test flasks. Three replicate flasks were run for each sample, under conditions of temperature and darkness already specified for samples without chloroform. The bottles were capped with aluminum foil and were sampled for DRP after approximately 1, 7, and 14 days of storage, by removal of a well-mixed 20 ml aliquot for filtration. Additional one-ml portions of chloroform were added as necessary to maintain saturation during the dark incubations.

In some of the incubations, the presence of phosphatase activity in the samples was determined by addition of 100 or 200 μgP/l as sodium tripoly phosphate (TPP) to a test flask containing 100 ml of chloroformed sample. The volume of the TPP spike was usually only 1 ml, so no correction was made for the small change in volume caused by the TPP spike. Table 26 shows the expected hydrolysis of TPP by the Murphy-Riley color reagent in the DRP analysis at ambient room temperature (about 27°C).

Table 26. HYDROLYSIS OF SODIUM TRIPOLY PHOSPHATE (TPP) BY MURPHY-RILEY COLOR REAGENT AT 27°C

Time	DRP in 10,000 μgP/1 TPP solution	Percent of P in TPP released by hydrolysis
	(μgP/1)	
15 min.	139	1.39
30 min.	174	1.74
45 min.	228	2.28
19 hr.	3850	38.5

In the 30-minute time period normally required for complete color development, only 1.7 percent of the TPP was hydrolyzed in the acidic color reagent (4 ml) plus sample (20 ml) mixture. The hydrolysis of TPP in chloroformed distilled (not autoclaved) water over 14 days was found to average 10 percent of the TPP present (Table 27).

Table 27. HYDROLYSIS OF 200 μgP/1 TPP IN CHLOROFORMED DISTILLED WATER AT 20°C

	DRP (μgP/1) after:		
Bottle No.	1 day	7 days	14 days
1	2	12	32
2	2	14	15
3	2	11	13
Mean values	2	12	20

No interferences from the chloroform were noted in the analysis of DRP, since in all cases the filtrates were undersaturated with respect to chloroform due to the vacuum filtration. Also, standards run in the same manner as the samples did not appear to differ from the standards run without chloroform.

In all dark incubations run before April 1, 1973, the test flasks were acid washed (1:1 HCl:water) before adding the sample, with no pretreatment of the flasks. Later incubations were run after the glassware had been pre-rinsed with sample water. Table 28 shows the recovery of orthophosphate in distilled water solutions incubated with anion-exchange resin. Three bottles with resin were incubated, and one bottle was analyzed at each time indicated. Recoveries were in the range of 92 to 98 percent, although a recovery of 92 percent at 200 μgP/l represented a loss of 16 μgP/l, probably sorbed on the glass walls of the test bottle. In a similar test (Table 29), 100 ugP/l orthophosphate solutions in chloroformed water were incubated for 14 days. Recoveries of 99 to 101 percent were found in this test. In both of these tests, the glassware was pre-rinsed with the test solution before incubation. Although losses from distilled water systems may not be the same as losses from samples, these results indicate that available P measured in long-term tests may be underestimates of the P actually released.

The possible sorption of orthophosphate by the plastic cubitainers used for sample transport and storage was also tested, as shown in Table 30. At the 50 μgP/l level, the sorption of orthophosphate from distilled water solution appeared to be less than 6 percent after 50 days in a cubitainer washed either with HCl and water, or with water alone. Since the HCl-water method was used to clean all cubitainers before use on sampling trips, Table 30 indicates that losses of DRP to the walls of the container should not be significant.

Table 28. DARK INCUBATION OF STANDARD 200 μgP/1 ORTHOPHOSPHATE SOLUTIONS WITH ONE GRAM OF DOWEX 1-X8 RESIN AT 20°C

	DRP on Resin (μgP/1)[a]		
	Bottle No. 1 (stored 13 days)	Bottle No. 2 (stored 27 days)	Bottle No. 3 (stored 50 days)
	190	195	185
	190	190	182
	---	201	---
mean values	190	195	184
% of added P recovered	95	98	92

---

[a] Values given are replicate analyses of the Na_2SO_4 resin leachate from each bottle, corrected for P recovery in the leaching procedure (see Figure 4). Supernatant DRP in the test bottles was undetectable.

Table 29. DARK INCUBATION OF CHLOROFORMED STANDARD 100 μgP/1 ORTHOPHOSPHATE SOLUTIONS AT 20°C

	DRP Concentration (μgP/1)		
Bottle No.	Initial	+$CHCl_3$ 7 days	+$CHCl_3$ 14 days
1	100	100	101
2	100	97	101
3	100	99	101
4	100	99	99

Table 30. EFFECT OF STORAGE TIME IN ONE-GALLON CUBITAINERS ON THE DRP CONCENTRATIONS OF DISTILLED WATER SOLUTIONS

Test A: Cubitainer rinsed with 6N HCl, followed by six rinses with distilled water before storage

Test B: Cubitainer rinsed six times with distilled water before storage

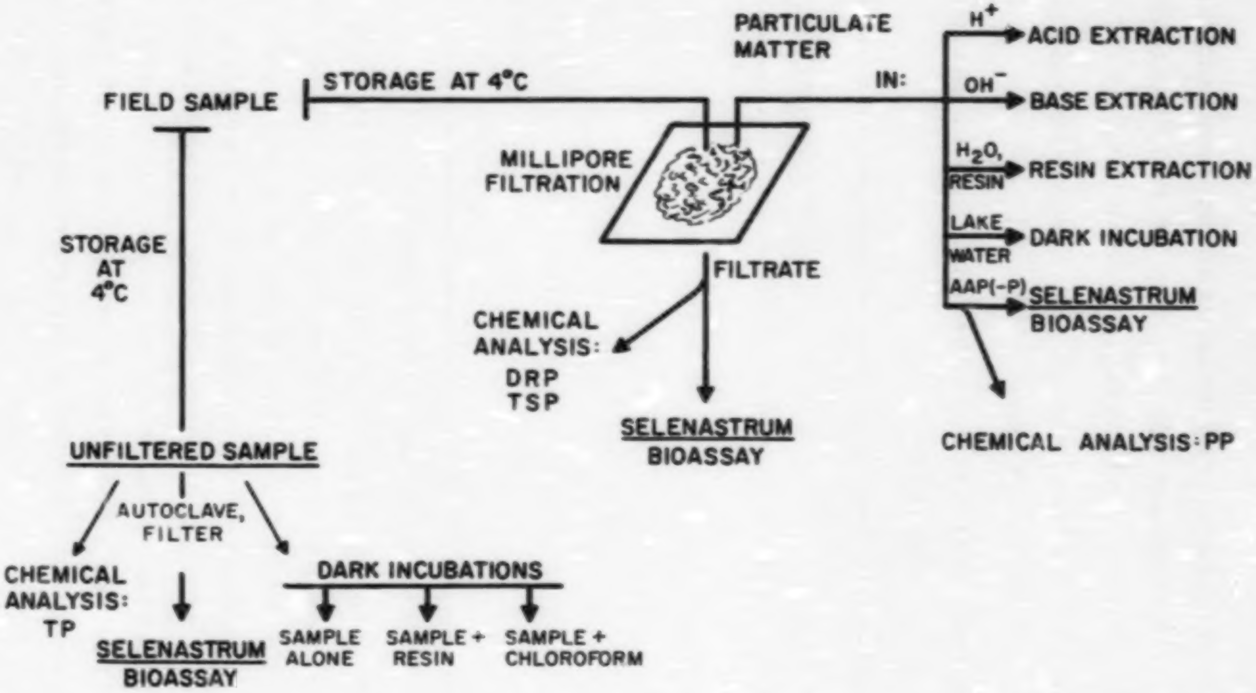
Both tests: Volume of solution = 2.5 l, stored at 4° in darkness

Time of Storage (Days)	DRP Concentration[a]	
	Test A	Test B
0	51	52
1	51	52
9	51	53
13	50	50
42	50	49

[a] Mean of duplicate determinations

A general summary of the procedures used in the handling and analysis of samples is given in Figure 14. Not all of the unit processes shown were attempted for each sample, because of difficulties in scheduling tests on samples arriving at random times in the laboratory. However, an attempt was made to use at least two different procedures for estimates of available P in particulate matter or in unfiltered river water samples.

Figure 14. Procedures in handling and analysis of samples


FIELD SAMPLE
STORAGE AT 4°C
STORAGE AT 4°C
MILLIPORE FILTRATION
PARTICULATE MATTER
IN:
H+
ACID EXTRACTION
OH−
BASE EXTRACTION
H2O, RESIN
RESIN EXTRACTION
LAKE WATER
DARK INCUBATION
AAP(−P)
SELENASTRUM BIOASSAY
CHEMICAL ANALYSIS: PP
FILTRATE
CHEMICAL ANALYSIS: DRP TSP
SELENASTRUM BIOASSAY
UNFILTERED SAMPLE
AUTOCLAVE, FILTER
CHEMICAL ANALYSIS: TP
SELENASTRUM BIOASSAY
DARK INCUBATIONS
SAMPLE ALONE
SAMPLE + RESIN
SAMPLE + CHLOROFORM

SECTION VII

RESULTS

In this chapter the results of chemical and biological tests of phosphorus availability are summarized. The data have been divided into four major sections, based on the origin of the water samples:

Madison Urban Runoff
Madison and New York Precipitation
Genesee River Basin Samples
New York River Samples

The Madison urban runoff samples were studied for the purpose of developing the techniques necessary for analyzing samples collected from New York rivers and runoff in connection with the International Field Year on the Great Lakes (IFYGL). In addition, the results of the Madison runoff studies were intended for comparison with the results from the urban runoff samples collected in the Genesee River basin.

Within each major section, the phosphorus forms in the samples are reported first, followed by the results of PP, TSP, and/or TP availability tests. Since the primary objective of these tests was to determine the fraction of these forms which might be available to algae, the results of all the tests are reported in relative terms, as percentages of the PP, TSP, and/or TP in the samples.

MADISON URBAN RUNOFF

Phosphorus Forms

The concentrations of analytically defined phosphorus forms in Madison urban runoff samples are given in Table 31. Total phosphorus (TP) levels of 59 to 2930 μgP/l were found in the samples, and particulate phosphorus (PP) forms represented 13 to 97 percent of sample TP. Since these samples were taken as grab samples during runoff events, the concentrations in Table 31 should not be taken as representative of average concentrations in runoff from a particular land usage or time of year. Rather, the data were obtained for investigation of the availability of soluble or particulate P in representative samples.

Chemical Extractions of PP

The concentrations of inorganic P extracted from particulate forms (extractable PP_i) by acid, base, or anion-exchange resin are presented in Table 32. By comparison with the total PP concentration in each sample, the PP_i extracted by each reagent was expressed as a percent of sample PP. All samples from a given land use class (see Table 9) were grouped together in Table 32, and a mean value was computed for each group and extraction method. These group mean values are displayed graphically in Figure 15. The ranges shown for each group and extraction method were taken from the data in columns six through eight of Table 32.

An important feature of Figure 15 is the relative similarity seen between the various land uses, for any given chemical extraction method. For example, the group mean values for acid extractable PP_i all fall within a range of 33 to 46 percent of PP. The corresponding range for base extractions is 22 to 27 percent, and for resin extractions 13 to 17 percent. The relative order of extraction yield

Figure 15. Percent of PP extracted from Madison urban runoff particles by chemical methods

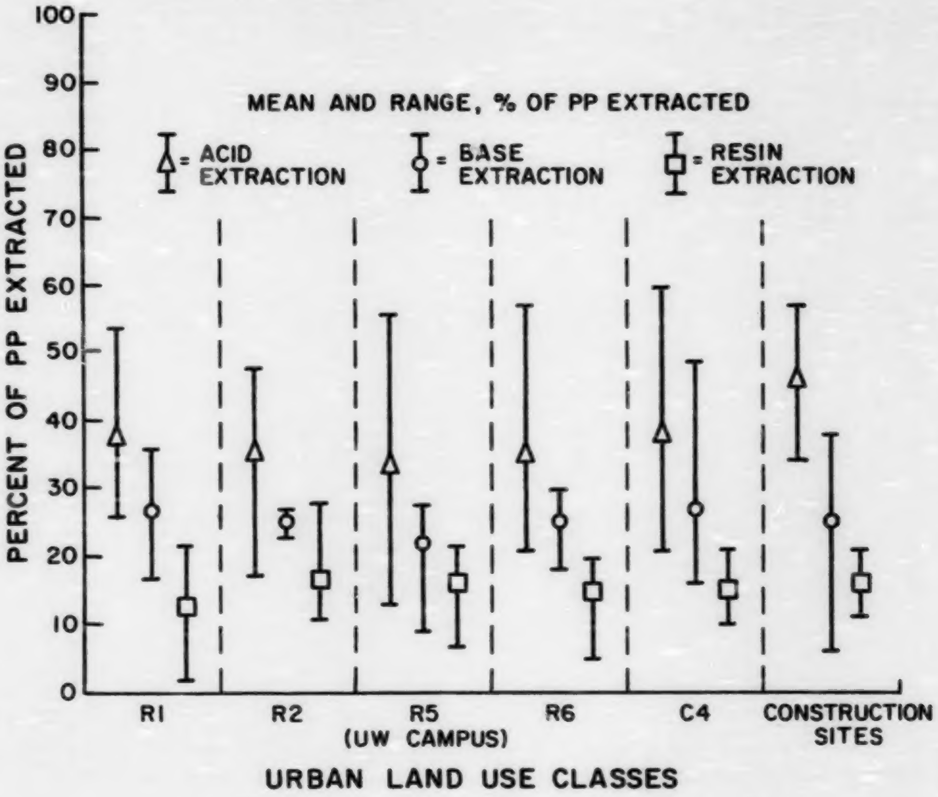


Table 31. PHOSPHORUS FORMS IN MADISON URBAN RUNOFF SAMPLES

Sample	Date Collected	Phosphorus (μgP/l)[a] DRP	TSP	TP	PP[b]
A-1	Aug. 11, 1972	80	87	201	114
B-1	"	144	163	290	127
D-1	"	94	105	291	186
A-2	Aug. 19, 1972	134	143	203	60
B-2	"	162	183	282	99
D-2	"	205	230	353	123
E-2	"	37	45	59	14
A-3	Aug. 23, 1972	80	85	118	33
B-3	"	112	119	233	114
D-3	"	168	180	679	499
E-3	"	41	45	74	29
A-4	Sept. 19, 1972	186	195	283	88
B-4	"	240	260	438	178
F-4	"	77	78	2930	2850
D-5	Sept. 20, 1972	219	251	433	182
G-5	"	33	41	131	90
A-6	Oct. 20, 1972	748	899	1030	130
B-6	"	1430	1640	2060	420
D-6	"	213	418	711	293
E-6	"	195	202	249	47
F-6	"	161	202	1090	890
H-6	"	415	451	539	88
A-7	Oct. 22, 1972	119	120	172	52
B-7	"	576	580	735	155
D-7	"	151	167	252	85
E-7	"	45	46	73	27
F-7	"	300[c]	280	645	365
H-7	"	74	77	146	69
A-8	Dec. 30, 1972	354	366	589	223
B-8	"	410	435	699	264
D-8	"	328	352	696	344
A-9	Jan. 17, 1973	868	979	1180[d]	203[e]
B-9	"	812	900	1110[d]	210[e]
F-9	"	486	552	755[d]	203[e]
D-10	Jan. 18, 1973	244	272	740[d]	468[e]
E-10	"	33	41	136[d]	95[e]
H-10	"	110	133	299[d]	166[e]
D-11	Feb. 1, 1973	252	257	698	441
E-11	"	30	34	179	145
H-11	"	110	115	377	262
A-12	Mar. 5, 1973	264	275	489	214
B-12	"	347	353	774	421
D-12	"	247	257	818	561
I-12	"	30	46	1460	1410

[a] Mean values from duplicate or triplicate analyses

[b] Unless otherwise noted, PP was determined by calculation (TP-TSP)

[c] This value of DRP was higher than the TSP, hence probably in error; the TSP value given was used to compute PP

[d] Determined by calculation from direct PP analysis (PP + TSP)

[e] Determined directly on (membrane) nonfiltrable particles

Table 32. EXTRACTION OF MADISON URBAN RUNOFF PARTICLES WITH ACID, BASE, AND ANION-EXCHANGE RESIN

Sample	PP (μgP/l)	PP_i (μgP/l) Extracted[a] Acid	Base	Resin	PP_i (% of PP) Extracted Acid	Base	Resin
		Residential (R1)					
A-1	114	--	31	16	--	27	14
A-2	60	--	20	1	--	33	2
A-3	33	--	5.8	6	--	17	17
A-4	88	31.8	19.8	8.8	36	22	10
A-6	130	62	38	21	48	30	16
A-7	52	16	11	2	30	21	4
A-8	223	121	80	49	54	36	22
A-9	203	75.1	56	29	37	28	15
A-12	214	55.1	--	39	26	--	18
		Group Mean Values			38	27	13
		Residential (R2)					
B-1	127	--	34	35	--	27	28
B-2	99	--	26	11	--	26	11
B-3	114	--	27	23	--	24	20
B-4	178	59.5	47.1	34	34	26	19
B-6	420	72	113	54	17	27	13
B-7	155	41	39	19	26	25	12
B-8	264	104	60	51	40	23	19
B-9	210	98.6	48	32	47	23	15
B-12	421	201	--	72	48	--	17
		Group Mean Values			35	25	17
		U. of Wis. Campus (R5)					
D-1	186	--	40	16	--	22	9
D-2	123	--	26	27	--	21	22
D-3	499	--	129	79	--	26	16
D-5	182	39	38	22	21	21	12
D-6	293	105	70	33	35	24	12
D-7	85	11	8.0	6	13	9	7
D-8	344	115	98	52	33	28	15
D-10	468	--	--	54.1	--	--	12
D-11	441	186	105	89	42	24	20
D-12	561	314	--	109	56	--	20
		Group Mean Values			33	22	16
		Residential (R6)					
H-6	88	27	22	16	26	26	18
H-7	69	15	13	3	21	18	5
H-10	166	--	--	26.7	--	--	16
H-11	262	150	78.9	53	57	30	20
		Group Mean Values			35	25	15

(Continued)

Table 32. EXTRACTION OF MADISON URBAN RUNOFF PARTICLES WITH ACID, BASE, AND ANION-EXCHANGE RESIN

	PP	PP$_i$ (µgP/l) Extracted[a]			PP$_i$ (% of PP) Extracted		
Sample	(µgP/l)	Acid	Base	Resin	Acid	Base	Resin
			Commercial (C4)				
E-2	14	--	5.1	3	--	37	21
E-3	29	--	14	6.0	--	49	21
G-5	90	37.9	16	12	42	18	13
E-6	47	13	9.7	4.5	28	20	10
E-7	27	5.5	4.3	3	21	16	10
E-10	95	--	--	14	--	--	15
E-11	145	87.4	35	23	60	24	16
			Group Mean Values		38	27	15
			Construction Sites				
F-4	2850	1580	155	301	55	6	11
F-6	890	357	262	148	40	30	17
F-7	365	124	137	76	34	38	21
I-12	1410	805	--	188	57	--	13
			Group Mean Values		46	25	16
F-9[b]	203	94.8	67	31	47	33	16

---

[a] Except for A-6 (base extract), all values are mean values of triplicate determinations; the A-6 base extract value is the mean value of duplicate determinations.

[b] This sample was collected from station F after the construction site had been sodded to prevent erosion.

for the three methods was acid > base > resin, as shown by the group mean values in Figure 15.

The data from Sample F-9 were not included in the previously described averaging processes, as this sample was not readily classified into one of the land use classes shown. Sample F-9 was collected from a construction site which had been covered with sod before sample collection. In comparison, samples F-4, F-6, and F-7 were collected from the same site while it was "open" and seriously eroding. The changed character of the site was indicated by the relatively low concentration of PP in Sample F-9 (Table 31). However, the proportions of extractable PP_i were not significantly different between Sample F-9 and Samples F-4, F-6, and F-7.

Extraction of PP by Selenastrum

As a check on the chemical extraction results, several bioassays with Selenastrum were run on Madison urban runoff particles in AAP (-P) medium. The results of these assays are given in Table 33. Taken as a general group, the residential samples (R1-R6 zoning classes) exhibited PP availability of 23 to 45 percent. This is a range of sample means, from the bioassay data compiled in Tables B.1 to B.5, Appendix B. Unfortunately, not enough bioassay data were collected from the other land use classes for computation of their group availability ranges. In the commercial sample (E-11) and open construction site sample (I-12), the proportion of PP extracted by Selenastrum appeared to be lower than in the residential Samples. In contrast, sample F-9 from the "covered" construction site exhibited the highest proportion of PP available (55 percent) of all the samples tested.

Table 33. EXTRACTION OF MADISON URBAN RUNOFF PARTICLES BY SELENASTRUM IN ALGAL BIOASSAYS

Sample	PP Assayed[a] (μgP/l)	Apparent Available P Mean Value[c] (μgP/l)	Apparent Available P Std.deviation (μgP/l)	Apparent Available P Mean Value (% of PP)
Residential (R1)				
A-8	201[b]	60	3	30
A-9	185	65	8	35
A-12	179	80	11	45
Residential (R2)				
B-8	130[b]	37	5	28
B-9	97	39	4	40
B-12	189	59	6	31
U. of Wis. Campus (R5)				
D-8	157[b]	43	3	27
D-11	209	49	8	23
D-12	121	31	2	26
Residential (R6)				
H-11	469	142	6	30
Commercial (C4)				
E-11	254	19	6	8
Construction Sites				
I-12	356	58	5	16
F-9[d]	94	52	5	55

[a] Unless otherwise noted, all PP values were determined directly on aliquots of the assayed suspensions.

[b] These values were calculated by difference (TP-TSP) as shown in Table B.2 of Appendix B.

[c] Mean values of four to six replicate culture flasks.

[d] This sample was collected after the construction site had been sodded to prevent erosion.

Incubation of Runoff Particles in Receiving Waters

In an effort to describe the effect of runoff particles on the phosphorus chemistry of their receiving waters, a short-term dark incubation of (membrane) nonfiltrable particles was made in either Lake Mendota or Monona water. Anion-exchange resin was added to some of the test flasks in order to study the behavior of the particles in lake waters with low DRP concentrations.

Table 34 shows the DRP changes seen during the dark incubations. In flasks with resin, the DRP value reported in the table is the "resin-extractable" DRP (R-DRP), equal to the sum of the DRP bound to the resin and the DRP in the supernatant solution above the resin and natural particles. The net mean DRP contributed by the particles was obtained by subtraction of the appropriate mean lake water control DRP value from the mean DRP results in flasks with particles plus lake water. These net values are given in Table 35. In flasks without resin, the net DRP contributions were positive for sample D-11 particles but negative for particles from samples E-11 or H-11. Comparison tests with anion-exchange resin showed positive net DRP contributions from the particles to the resin. In the latter tests, the resin lowered the concentration of DRP in the receiving waters from 66 and 80 μgP/l in Lake Mendota and Monona waters, respectively, down to about 2 μgP/l. Comparison of the maximum net mean R-DRP values seen in these tests with the PP concentrations in the flasks (Table 35) gave the following percents of PP contributed by the particles:

Sample particles	Maximum percent of PP extractable by resin
D-11	21
E-11	8
H-11	10

Table 34. DRP CHANGES DURING DARK INCUBATION OF RUNOFF PARTICLES IN LAKE WATER

	Initial	DRP (µgP/1) after incubation[b]			
	DRP[a]	Sample only		Sample + resin	
Test sample	(µgP/1)	13 days	26 days	13 days	26 days
L. Mendota water	66	82	88	74	77
		87	88	75	78
		84	89	73	80
mean values		84	88	74	78
100 ml L. Mendota water	66	88	94	98	101
+ particles from 25 ml		90	98	102	98
D-11		91	99	96	97
mean values		90	97	99	99
L. Monona water	80	96	96	97	94
		103	104	94	95
		100	104	94	95
mean values		100	101	97	96
100 ml L. Monona water	80	69	79	105	108
+ particles from 100 ml		70	82	103	105
E-11		71	81	105	112
mean values		70	81	104	108
100 ml L. Monona water		63	86	122	126
+ particles from 100 ml		67	87	115	138
H-11		65	89	115	126
mean values		65	87	117	130

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[a] Dark Incubation was begun February 7, 1973.

[b] DRP in flasks with resin includes DRP in solution plus DRP on the resin.

Table 35. SUMMARY OF NET MEAN DRP RELEASED FROM RUNOFF PARTICLES TO LAKE WATER (See Table 34)

Runoff sample particles		PP from runoff (μgP/l)	Net Mean DRP (μgP/l) from Particles[a]			
			Sample only		Sample + resin	
			13 days	26 days	13 days	26 days
D-11 in L. Mendota water		116 118 119	6	9	25	21
	Mean	118				
E-11 in L. Monona water		162 159 153	-30	-20	7	12
	Mean	158				
H-11 in L. Monona water		335 322 330	-35	-14	20	34
	Mean	329				

---

[a] Calculated for a given incubation time and treatment ± resin by subtracting mean DRP values for lake water samples from mean DRP values from samples with lake water + particles.

The relationship between orthophosphate, resin, and runoff was also investigated by spiking sample D-11 with orthophosphate, as shown in Table 36. The samples were shaken for 24 hours after spiking, then were analyzed for DRP. The samples, including the unspiked control, demonstrated phosphorus uptake (Table 36-a) of 16 to 41 μgP/l. To see if the phosphorus lost to the particles could be recovered with anion-exchange resin, one gram of resin was added to each flask, which was then shaken continuously for another 24 hours. The total DRP recovered by the resin is given in part (b) of Table 36. Comparison of these values with the expected DRP concentrations in the flasks after spiking (Table 36 -c) showed recoveries of 100 to 111 percent.

Dark Incubations of Unfiltered Runoff

Samples of unfiltered runoff were stored in darkness for up to 50 days, with anion-exchange resin added to some of the samples as a sink for any phosphorus released from the runoff particles. The data from these tests are compiled in Tables C.1 to C.6 of Appendix C. Comparison of the resin-extractable DRP (R-DRP) values to the DRP values in flasks without resin generally showed the R-DRP levels to be approximately equal to or greater than the DRP levels in flasks without resin. In the resin flasks, the R-DRP values at 50 days were generally close to the 25-day incubation values in the early tests. Consequently, later tests were carried out for only about 26 days of incubation.

Table 37 lists the maximum observed R-DRP values for each sample incubated. As an estimate of the contribution of inorganic P to the resin from the particulate matter in the samples, the following equation (1) was employed:

Table 36. RECOVERY OF INORGANIC P FROM SPIKED URBAN RUNOFF SAMPLE D-11 BY ANION-EXCHANGE RESIN EXTRACTION

A. DRP 24 hours after spiking 100 nl of sample D-11 (Initial sample DRP = 212 µgP/l)

	DRP (µgP/l)		
Flask code[a]	Added in spike	Expected conc.	Observed conc.
A	0	212	191
B	50	262	246
C	98	310	281
D	192	404	363

B. DRP after equilibration of 80 ml of spiked sample with anion-exchange resin for 24 hours

	DRP (µgP/l)		
Flask code	In solution	On resin	Total recovered
A	8	205	213
B	11	269	280
C	12	332	344
D	14	414	428

C. Recovery of expected DRP by resin equilibration

	DRP (µgP/l)		%
Flask code	Expected	Recovered	Recovered
A	212	213	100
B	262	280	107
C	310	344	111
D	404	428	106

[a] Each code letter represents four replicate test flasks: all DRP values are mean values of the four flasks.

Table 37. MAXIMUM DRP VALUES IN TEST FLASKS WITH ANION-EXCHANGE RESIN DURING DARK INCUBATIONS OF MADISON URBAN RUNOFF

Sample	Initial concentration DRP (µgP/l)	Initial concentration TSP (µgP/l)	Incubation period (days)	Max.obs. R-DRP[a] in incubation (µgP/l)	Inorganic P released from PP (Max. observed values) (µgP/l)	Inorganic P released from PP (Max. observed values) (% of PP)[e]
			Residential (R1)			
A-8	354	366	50	410	44	20
A-9	868	979	50	970	-9	-4
A-12	264	275	21	311	36	17
			Residential (R2)			
B-4	240	260	50	318	58	33
B-7	576	580	50	648	68	44
B-8	410	435	25	467	32	12
B-9	812	900	25	883	-17	-8
B-12	347	353	21	392	39	9
			U. of Wis. Campus (R5)			
D-8	328	352	50	422	70	20
D-10	244	272	50	342	70	15
D-11	252	257	26	316	59	13
D-12	247	257	21	336	79	14
			Residential (R6)			
H-11	110	115	26	105	-10	-4
			Commercial (C4)			
E-11	30	34	13	36	2	1
			Construction Sites			
F-7	300	280[c]	50	395	115	31
F-9[d]	486	552	50	556	4	2
I-12	30	46	21	142	96	7

---

[a] R-DRP = DRP in solution plus DRP bound to resin µgP/l of sample

[b] Calculated by: R-DRP - Initial sample TSP

[c] Used in calculation of DRP from PP, although it is a lower value than DRP

[d] This sample was collected after the construction was sodded to prevent erosion

[e] PP values used to calculate these data are given in Table 31.

$$(1)\quad \text{Estimated inorganic P released from PP} = \text{Maximum observed R-DRP} - \text{Initial Sample TSP}$$

Equation (1) is only an estimate because the difference between the initial sample DRP and TSP concentrations (dissolved unreactive-P) cannot be assumed to be converted completely to DRP by hydrolysis during the incubation, nor can the particles be assumed to contribute DRP equal to the entire difference between the maximum R-DRP observed and the initial sample DRP. However, it can be stated that *at least* the difference between the maximum observed R-DRP and the sample TSP had to come from the runoff particles. The last column in Table 37 expresses the estimated contributions from the particles in terms of percent of PP. The data show that in some samples (A-9, B-9, F-9, H-11, and E-11) the maximum R-DRP levels were similar to the initial TSP levels in the samples (values from Table 31). For these samples, the necessity of invoking a possible contribution of inorganic P from PP was unnecessary. In other samples, however, R-DRP exceeded TSP, and calculated values of 12 to 44 percent of PP were found for the estimated contribution of inorganic P from runoff particles.

Bioassay of Soluble P in Runoff

The results of algal bioassays of filtered runoff samples are given in Table 38. These data are compiled from Tables B.6 to B.10 of Appendix B and are based on uncorrected A_{750} data. Table 38 shows that the urban runoff filtrates generally showed high relative TSP availability. The results were compared to sample TSP, since TSP represented the maximum possible available P level. In several residential samples, the apparent percent of TSP available to *Selenastrum* exceeded 100 percent. One such sample, B-9 showed 206 percent of TSP available. Spikes of 50 and 100 μgP/l were added to this sample,

Table 38. BIOASSAY OF FILTERED URBAN RUNOFF SAMPLES WITH SELENASTRUM

Sample	P in assay flasks DRP	P in assay flasks TSP	Apparent algal-available P[d] in assay flasks Mean value	Std.dev.	Mean Value
	(µgP/l)	(µgP/l)	(µgP/l)	(µgP/l)	(% of TSP)
			Residential (R1)		
A-8	66	68	73	7	107
A-9	161	181	>200	--	>110
A-12	49	51	84	10	165
			Residential (R2)		
B-9[a]	150	167	>200	--	>120
B-9[b]	--	167	343	10	206
B-12	64	65	116	2	179
			U. of Wis. Campus (R5)		
D-8	61	65	74	6	114
D-10	45	50	57	1	114
D-11	47	48	47	4	98
D-12	46	48	71	7	148
			Residential (R6)		
H-10	81	98	46	4	47
H-11	20	21	12	3	57
			Commercial (C4)		
E-10	24	30	5	1	17
E-11	22	25	7	3	28
			Construction Sites		
I-12	22	34	12	1	35
F-9[c]	90	102	139	6	136

[a] Assayed Jan. 19, 1973

[b] Assayed Mar. 6, 1963

[c] This sample was collected after the construction site had been sodded to prevent erosion.

[d] Mean values based on uncorrected A_{750} data

producing the response curve shown in Figure 16. The growth response with the 50 μgP/l spike indicated that phosphorus was still limiting growth, although the next 50 μgP/l increment clearly showed a smaller growth response per unit of added P (see 100 μgP/l spike data point). If the response curve for the spiked sample is transposed to the chemically determined value of TSP (167 μgP/l) and compared to the standard curve drawn from AAP standard cultures, the reason for the overestimation of available P in the sample becomes readily apparent. The absorbances of the samples (which were spiked with AAP(-P) nutrients) were higher than the AAP standard curve shown in the figure. Thus, the apparent available P (arrows) was much higher than even the theoretical upper limit, TSP.

Of the 15 different samples tested, 10 showed available P of at least 98 percent of TSP. The five remaining samples had available P concentrations of less than 60 percent of TSP; their concentrations of available P were even less than the chemically measured DRP levels. Four of the samples with low soluble P availability came from commercial or high-density residential areas and one was from an open construction site.

PRECIPITATION SAMPLES

Madison Snow

Table 39 (a) shows the concentrations of P forms found in three samples of Madison snow collected April 10, 1973. DRP and TSP were essentially identical in these samples, and PP forms were clearly dominant. By filtering large volumes of melted snow, PP concentrations of 82 to 131 μgP/l were obtained for Selenastrum growth bioassays (Table B.11, Appendix B. As shown in Table 39 (b), less than 25 percent of the PP in the snow samples was available to Selenastrum in 18 days. The sample collected from the roof of the

Figure 16. Bioassay of soluble P in Madison urban runoff sample #B-9

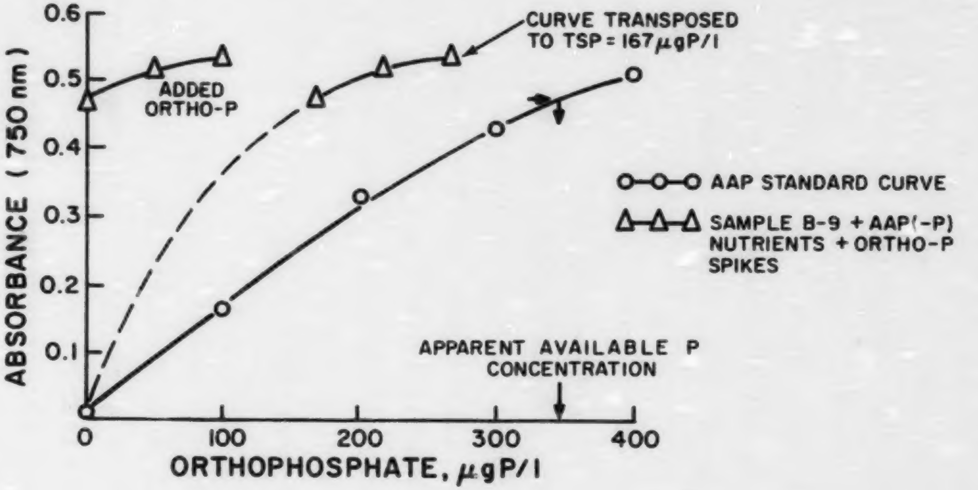

Table 39. PHOSPHORUS FORMS AND ALGAL-AVAILABLE PP IN MADISON SNOW SAMPLES COLLECTED APRIL 10, 1973

A. Phosphorus Forms

Sample	Sampling site	Phosphorus (μgP/l) DRP	TSP	TP	PP
1.	Picnic Point Park	3	2	32	30
2.	City-County Bldg. Roof	3	4	37	33
3.	Near Meat Packing Plant	3	0	44	44

B. Bioassay of PP Forms with *Selenastrum*

Sample	PP Assayed (μgP/l)	Apparent Available P Mean value (μgP/l)	Std.deviation (μgP/l)	Mean value (% of PP)
1.	82	14	4	17
2.	118	<2	-	<2
3.	131	30	3	23

City-County building in the commercial district of Madison did not show detectable growth in any of the culture flasks.

New York Rain Gage Samples

A summary of the P forms in samples from open and covered rain gages in the State of New York is given in Table 40. Unlike urban runoff these precipitation samples had very high TSP concentrations along with low DRP levels. (In most urban runoff samples, TSP and DRP were similar). Because of the small volumes of rain water and their low concentrations of PP, unfiltered samples were bioassayed to estimate the available fraction of TP rather than of PP. Bioassays of the unfiltered rain water (Table B.12, Appendix B) showed that in those samples with "abnormally" high TSP concentrations, available P was, like DRP, only a small fraction of TSP (Tables 41 and 40). In contrast, those samples with DRP close to TSP (June 601 and 604) had available P levels only 21 μgP/l or less lower than TSP.

On a TP basis, the data in Table 41 show that only three samples had available P concentrations which were 10 percent or more of TP (May 601, June 601, and June 604). Except for the June 604 sample, the available P in these three samples was lower than even the sample DRP values.

GENESEE R. BASIN SAMPLES

Phosphorus Forms

Although many samples were received from the Genesee R. basin in the State of New York, only those listed in Table 42 were selected for PP availability studies. The other samples were too dilute for such analyses. As shown in Table 42, the concentrations of PP in the samples studied varied from 17 to 2110 μgP/l and from 35 to 99 percent of TP.

Table 40. PHOSPHORUS FORMS IN NEW YORK RAIN GAGE SAMPLES

Sample location Code No.[b]	Month Collected	Phosphorus (μgP/l)[a]			
		DRP	TSP	TP	PP
601-O	May	60	245	420	175
602-C	"	8	62	86	24
603-C	"	< 1	2	4	2
604-C	"	< 1	2	6	4
605-C	"	< 1	304	350	46
606-C	"	1	401	439	38
601-C	June	52	63	72	9
602-C	"	4	6	10	4
603-C	"	5	82	106	24
604-C	"	48	58	64	6
605-C	"	< 1	6	9	3
606-C	"	1	6	12	6
608-O	"	1	318	346	28

---

[a] All values are mean values of two or three replicate analyses except May 601, 602, 603, and 604 TSP values, where single analyses are reported.

[b] The sample location code number refers to the EPA sampling site, as given in Table 10. The letters "C" and "O" refer to the condition of the rain gages during dry periods (Closed or Open).

Table 41. BIOASSAY OF UNFILTERED NEW YORK RAIN GAGE SAMPLES WITH SELENASTRUM

Sample	Sample TP (µgP/l)	Apparent Available P Mean value (µgP/l)[a]	Std.deviation (µgP/l)	Mean value (% of PP)
May-601-O	420	42	4	10
May-602-C	86	4	0.5	5
May-603-C	4	<14	-	-
May-604-C	6	<14	-	-
May-605-C	350	<14	-	<4
May-606-C	439	< 3	-	<1
June-601-C	72	42	4	58
June-602-C	10	<14	-	-
June-603-O	106	4	1	4
June-604-C	64	58	11	90
June-605-C	9	<14	-	-
June-606-C	12	<14	-	-
June-608-O	346	<14	-	<4

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[a] These values were calculated from Table B.12 of Appendix B by multiplying the bioassay results in Table B.12 by the sample dilution correction factor of 27/20.

Table 42. PHOSPHORUS FORMS IN GENESEE RIVER BASIN SAMPLES

		Phosphorus (μgP/l)[a]			
Sample	Date Collected	DRP	TSP	TP	PP
402-6	Oct. 6, 1972	72	77	118	41
402-8	Nov. 3, 1972	70	78	188	110
404-8	Nov. 2, 1972	182	193	350	157
407-8	"	27	29	361	332
409-8	"	1	8	131	123
402-9	Nov. 15, 1972	55	66	112	46
409-9	Nov. 14, 1972	14	26	2140	2110
502-1	Dec. 15, 1972	27	33	60	27
502-7	Mar. 22, 1973	24	26	69	43
507-7	"	--	4	39	35
502-8	April 4, 1973	19	27	59	32
504-8	"	54	60	452	392
507-8	April 3, 1973	6	4	29	25
507-9	April 17, 1973	2	2	27	25
502-10	May 1, 1973	15	22	77	55
502-11	May 16, 1973	37	46	81	35
507-11	~~May~~ 15, 1973	2	5	22	17
501-12	May 30, 1973	3	12	60	48
502-12	"	43	55	165	110
507-12	"	2	4	32	28
501-13	June 12-13, 1973	1	4	31	27
507-13	"	5	9	239	230
501-14	June 26, 1973	6	7	38	31
502-14	"	59	66	129	63
507-14	June 25, 1973	9	10	284	274

[a] Mean values of duplicate or triplicate determinations

Chemical Extractions of PP

The concentrations of inorganic P extracted from PP forms (extractable PP_i) by acid, base, and resin are given in Table 43. These concentrations were compared to sample PP concentrations and expressed as a percent of PP, then averaged as a group on the basis of sampling station. Stations No. 1, 4, 7, and 9 showed group mean values for acid extractable PP_i in the range of 22 to 30 percent, while the corresponding value for Station No. 2 was 48 percent. Figure 17 shows that in general the group mean values for the base and resin extractions of all stations other than No. 2 were also lower than the corresponding values for Station No. 2. For any given station, the relative yields from the chemical extraction methods were in the order: acid > base > resin. The ranges shown for each group and extraction method were taken from the data in columns six through eight of Table 43.

Extraction of PP by Selenastrum

Generally, less than 25 percent of the PP incubated in AAP(-P) medium was available to *Selenastrum* in 18 days, even if the particulate suspensions were autoclaved before the bioassays (Table 44). Only one value over 25 percent was recorded (34 percent), in a bioassay run after autoclaving the particles from Sample No. 502-14. These bioassay data are compiled from Tables B.13 to B.28, Appendix B.

Dark Incubations of Unfiltered Samples

Table 45 shows the data collected from very limited dark incubations of Genesee R. basin samples, as summarized from Tables C.2 to C.12, Appendix C. The R-DRP values in all three of the samples tested were greater than the sample TSP values. Calculated estimates of the inorganic P contribution from PP ranged from 7 to 28 percent of PP.

Figure 17. Percent of PP extracted from Genesee R. basin particles by chemical methods

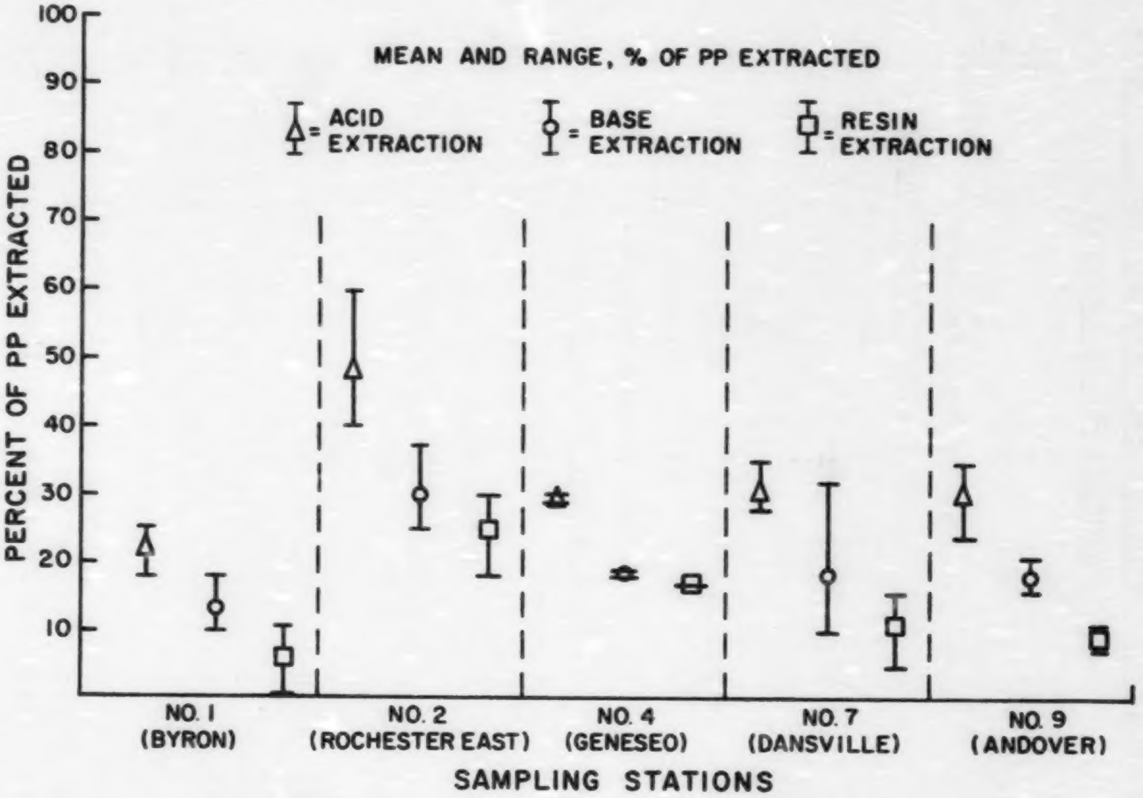

Table 43. EXTRACTION OF GENESEE R. BASIN SAMPLE PARTICLES WITH ACID, BASE, AND ANION-EXCHANGE RESIN

Sample	PP (µgP/1)	PP_i (µgP/1) Extracted[a]			PP_i (% of PP) Extracted		
		Acid	Base	Resin	Acid	Base	Resin
Station No. 1 (Byron)							
501-12	48	11.9	5.1	0.5	25	11	1
501-13	27	4.8	5.0	2	18	18	7
501-14	31	6.9	3.0	3	22	10	11
		Group Mean Values			22	13	6
Station No. 2 (Rochester East)							
402-6	41	17.3	11	11	42	28	26
402-8	110	43.8	32.0	27	40	29	25
402-9	46	27.7	17	10	60	36	23
502-7	43	--	15.9	12	--	37	29
502-8	32	--	8.0	5.6	--	25	18
502-11	35	16.1	10	10	46	30	30
502-12	110	53.5	29.6	30	49	27	27
502-14	63	32.7	17	16	52	27	25
		Group Mean Values			48	30	25
Station No. 4 (Genesseo)							
404-8	157	46	29	27	30	18	17
504-8	392	112	75	69	29	19	17
		Group Mean Values			30	18	17
Station No. 7 (Dansville)							
407-8	332	116	60	35	35	18	11
507-7	35	11.8	--	2	34	--	5
507-8	25	--	8.0	1.9	--	32	8
507-9	25	7.3	2.6	3	29	10	13
507-11	17	4.8	2.7	2.8	28	16	16
507-12	21	6.2	5	3	29	22	16
507-13	230	64.5	34	31	28	15	13
507-14	274	82.8	28.7	15	30	11	5
		Group Mean Values			30	18	11
Station No. 9 (Andover)							
409-8	123	28.6	20	9	24	16	7
409-9	2110	742	445	228	35	21	11
		Group Mean Values			30	18	9

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[a] Mean values of duplicate or triplicate determinations

Table 44. EXTRACTION OF GENESEE R. BASIN SAMPLE PARTICLES BY SELENASTRUM IN ALGAL BIOASSAYS

Sample	PP Assayed[a] (μgP/l)	Apparent Available P		
		Mean value (μgP/l)[b]	Std.deviation (μgP/l)	Mean value (% of PP)
		Station No. 1 (Byron)		
501-12	146	< 4	--	< 3
501-13(A)	103	17	0.4	16
501-14	52	< 5	--	<10
501-14(A)	54	11	0.8	20
		Station No. 2 (Rochester East)		
502-1[c]	43	9	5	21
502-1[d]	46	10	5	22
502-7	137	30	5	22
502-8	100	21	5	21
502-10	328	79	31	24
502-11	164	< 2	--	< 1
502-12	81	< 6	--	< 7
502-14	127	< 2	--	< 2
502-14(A)	128	44	2	34
		Station No. 4 (Genesseo)		
504-8	57	4	0.7	7
		Station No. 7 (Dansville)		
507-7	61	0	--	< 3[e]
507-8	64	2	1	3
507-11	68	< 2	--	< 3
507-12	210	< 3	--	< 1
507-13(A)	224	17	2	8
507-14	444	12	2	3
507-14(A)	481	50	2	10

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[a] Unless otherwise noted, these values were determined directly on aliquots of the assayed suspensions.

[b] Mean values of four to six replicate culture flasks

[c] Assayed on Dec. 21, 1972

[d] Assayed on Jan. 9, 1973; PP was calculated by difference (TP-TSP), as shown in Table B.2 of Appendix B.

[e] Calculated assuming a minimum detectable P concentration of 2 μgP/l

(A) = Autoclaved suspensions of particles

Table 45. MAXIMUM DRP VALUES IN TEST FLASKS WITH ANION-EXCHANGE RESIN DURING DARK INCUBATIONS OF GENESEE R. BASIN SAMPLES

Sample	Initial Concentration DRP	Initial Concentration TSP	Incubation Period	Max.obs. R-DRP[a] in incubation	Inorganic P released from PP[b] (max.observed values)	
	(µgP/l)		(days)	(µgP/l)	(µgP/l)	(% of PP)[c]
402-8	70	78	50	109	31	28
407-8	27	29	50	104	75	22
507-13	5	9	50	26	17	7

[a] R-DRP = DRP in solution plus DRP bound to resin (µgP/l of sample)

[b] Calculated by: R-DRP - Initial sample TSP

[c] PP values were taken from Table 42 for this computation.

NEW YORK RIVER SAMPLES

Phosphorus Forms

The phosphorus forms measured in samples of New York tributaries to Lake Ontario and in samples of lake water collected near the mouths of the Genesee and Oswego Rivers are given in Table 46. PP in the samples ranged from 5 to 360 μgP/l and from 23 to 94 percent of TP. Emphasis was placed on the estimation of the algal-available fraction of both PP and TP in all studies with river water samples.

Chemical Extractions of Genesee R. PP

The results of chemical extractions of Genesee R. particles are reported in Table 47. The PP_i extracted by acid was in all cases more than the PP_i extracted by base or resin. However, the range of the percent of PP extracted by acid was quite broad, from 21 to 79 percent. The combined range for base and resin extractions was less broad, from 6 to 31 percent, with close agreement between the results of the two methods. Table 48 shows the data collected from a dark incubation of Sample No. 42 (Genesee R.) particles in Lake Ontario water of initial DRP equal to 19 μgP/l. In test flasks with resin, a maximum net value of 18 percent of PP was extracted by the resin after 24 days of dark incubation. The corresponding maximum value for flasks without resin was 14 percent of PP. Both values are similar to the values noted for base and resin extractable PP_i in Sample No. 42 (Table 47).

Extraction of PP by Selenastrum

Membrane-nonfiltrable particles from New York rivers were bioassayed in AAP(-P) medium both in their natural forms or after autoclaving. Table 49 shows that natural particles did not yield more than 6 percent of their phosphorus to Selenastrum cells for growth. The presence of several species of natural algae was noted in many of the samples during the hemocytometer cell counting of the

Table 46. PHOSPHORUS FORMS IN NEW YORK RIVER WATER SAMPLES AND LAKE ONTARIO RIVER MOUTH SAMPLES

Sample No.	River	Date Collected	Phosphorus (µgP/l)[a]			
			DRP	TSP	TP	PP[b]
16	Genesee	Aug. 2, 1972	43	82	167	85
17	Genesee R. Mouth	"	12	20	43	23
22	Oswego R. Mouth	Aug. 7, 1972	58	62	93	31
23	Oswego	"	49	58	96	38
24	Oswego R. Mouth	Sept. 1, 1972	41	50	88	38
25	Black	Aug. 28, 1972	14	19	53	34
26	Oswego	"	79	87	154	67
27	Niagara (Ft. Niagara)	Feb. 26, 1973	4	8	18	10
28	Oswego	March 2, 1973	68	71	93	22
29	Oswego	March 12, 1973	78	82	106	24
31	Oswego	March 28, 1973	43	49	95	46
32	Niagara (Beaver I. Park)	April 6, 1973	2	5	30	25
33	Niagara (Ft. Niagara)	"	5	10	34	24
34	Genesee	April 7, 1973	26	26	386	360
35	Oswego	"	47	52	105	53
36	Black	"	7	12	34	22
40	Niagara (Beaver I. Park)	April 30, 1973	6	10	15	5
41	Niagara (Ft. Niagara)	"	4	8	22	14

(Continued)

Table 46. PHOSPHORUS FORMS IN NEW YORK RIVER WATER SAMPLES AND LAKE ONTARIO RIVER MOUTH SAMPLES

Sample No.	River	Date Collected	Phosphorus (μgP/l)[a]			
			DRP	TSP	TP	PP[b]
42	Genesee	May 1, 1973	40	45	150	105
43	Oswego	"	38	46	96	50
44	Black	"	9	15	34	19
47	Oswego	May 14, 1973	41	50	98	48
49	Niagara (Beaver I. Park)	May 27, 1973	2	6	51	45
50	Niagara (Ft. Niagara)	"	1	7	26	19
51	Genesee	May 28, 1973	104	111	173	62
52	Oswego	"	50	56	104	48
53	Black	"	5	16	41	25
54	Oswego	May 31, 1973	40	51	87	36
55	Oswego	June 4, 1973	35	45	96	51
56	Niagara (Ft. Niagara)	June 16, 1973	26	33	59	26
57	Niagara (Beaver I. Park)	"	3	7	86	79
58	Genesee	June 17, 1973	49	58	204	146
59	Oswego	"	46	59	147	88
60	Black	"	13	24	99	75

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[a] All values are mean values of triplicate determinations except Sample No. 17 and No. 18 DRP values, which are mean values of duplicate determinations

[b] Determined by difference (TP-TSP)

Table 47. EXTRACTION OF GENESEE RIVER PARTICLES WITH ACID, BASE, AND ANION-EXCHANGE RESIN

Sample No.	PP[a] (μgP/l)	PP_i (μgP/l) Extracted			PP_i (% of PP) Extracted		
		Acid	Base	Resin	Acid	Base	Resin
34	360	284	41	32	79	11	9
42	108[b]	59	19	25	55	18	23
51	62	27	17	19	44	28	31
58	146	30	17	8	21	12	6

[a] Unless otherwise noted, these values were determined by calculation (TP-TSP)

[b] Determined directly on a suspension of particles in distilled water

Table 48. DRP CHANGES DURING DARK INCUBATION OF GENESEE RIVER SAMPLE NO. 42 PARTICLES IN LAKE ONTARIO WATER

	Initial DRP	DRP(µgP/1) after incubation					
		Sample Only			Sample + resin		
Test sample	(µgP/1)[a]	13 days	24 days	50 days	13 days	24 days	50 days
L. Ontario water	19	2	2	1	5	4	1
		3	2	1	5	4	4
		7	1	1			
	Mean values	4	2	1	5	4	2
100 ml L. Ontario water + particles[b] from 100 ml Sample No.42	19	17	11	10	22	24	10
		19	13	1	22	20	16
		19	15	1			
	Mean values	18	13	4	22	22	13
Net Mean values (DRP from No.42 particles)		14	11	3	17	18	11

[a] The dark incubation was begun May 7, 1973

[b] The PP contributed to the test flasks by Sample No. 42 particles was 101 µgP/1; therefore, the Max. % of Sample No. 42 PP found as DRP = 18/101 X 100 = 18% in flasks with resin, and 14/101 X 100 = 14% in flasks without resin

Table 49. EXTRACTION OF NEW YORK RIVER WATER PARTICLES BY *SELENASTRUM* IN ALGAL BIOASSAYS

Sample No.	PP Assayed[a] (µgP/l)	Apparent Available P		
		Mean value (µgP/l)	Std.deviation (µgP/l)	Mean value (% of PP)
		Niagara R. at Ft. Niagara		
50	79	<4	--	<5
50(A)	79	45	4	57
56(A)	87	29	2	33
		Genesee R.		
34	119	3	1	2
42	187	<12	--	<6
51	115	<3	--	<3
51(A)	115	47	4	41
58(A)	237	85	18	36
		Oswego R.		
43	309	<2	--	<1
47	236	<5	--	<2
52	141	<3	--	<2
52(A)	141	62	6	44
59(A)	163	52	5	32
		Black R.		
44	133	4	1	3
53	66	<3	--	<5
53(A)	66	30	7	45
60(A)	189	49	5	26

---

[a] Determined directly on aliquots of suspensions of particles in AAP(-P) medium

(A) = Autoclaved suspensions of particles cultures. In one case (Sample No. 58), the Selenastrum could not be counted because the cells formed clumps with the natural algae.

In contrast to the relatively low availability of PP in natural particles, the autoclaved particles yielded from 26 to 57 percent of their PP to Selenastrum. These data were taken from Tables B.13 to B.28, Appendix B.

Total P Availability

Unfiltered river water samples were treated by three different procedures, in an effort to estimate the percent of TP which might become available in Lake Ontario.

Chloroform addition--

Table 50 shows the changes in DRP which occurred in Sample No. 55 (Oswego R.) as a result of chloroform addition and subsequent dark incubation. After only one day, the DRP concentration was increased from 35 μgP/l to 51 μgP/l. Since 51 μgP/l was greater than the initial TSP value of 45 μgP/l, some of the DRP increase must have come from the PP in the sample. Taking the highest mean value observed (59 μgP/l) as an estimate of the potential available P in the sample, the estimated DRP contribution from the 51 μgP/l of PP in the sample was calculated as 59-45=14 μgP/l, or 14/51 X 100 percent of PP (27 percent). The test flasks with 100 μgP/l of a condensed phosphate spike, in the form of sodium tripoly phosphate (TPP), showed complete hydrolysis of TPP to DRP in seven days (160 μgP/l DRP, compared to 59 μgP/l in the controls). That this hydrolysis was probably due to natural enzymes in the river water was shown by the relatively slow hydrolysis rate seen in the "chemical hydrolysis" control flasks containing 100 μgP/l TPP in chloroformed, distilled (not autoclaved) water; only 12 percent of the TPP was hydrolyzed after seven days.

Table 50. DRP CHANGES IN A CHLOROFORMED OSWEGO R. SAMPLE (NO. 55), WITH AND WITHOUT ADDED SODIUM TRIPOLY PHOSPHATE (TPP)

A. Initial sample No. 55 data: DRP = 35 μgP/l
TSP = 45 μgP/l
TP = 96 μgP/l

B. Dark incubation with chloroform[a]

Test sample	Flask No.		DRP (μgP/l) after incubation 1 day	7 days
No. 55 water	1		49	59
	2		52	57
	3		51	62
		Mean values	51	59
No. 55 water + TPP	1		75	146
	2		76	169
	3		79	166
		Mean values	77	160
Dist. water + TPP	1		2	12
	2		2	14
	3		2	11
		Mean values	2	12

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[a] Test flasks were prepared as follows:
3 flasks with 200 ml of No. 55 + 2 ml $CHCl_3$
3 flasks with 200 ml of No. 55 + 2 ml $CHCl_3$ + 2 ml of 10 mgP/l sodium tripoly-P (TPP)
3 flasks with 200 ml of dist. water + 2 ml $CHCl_3$ + 2 ml of 10 mgP/l sodium tripoly-P (TPP)

Table 51 summarizes the results of tests with river and river mouth samples which had been stored at 4°C for 16 to 30 days prior to the chloroform addition. Since the objective of these tests was to estimate the long-term potential available P in the river waters, any biological changes occurring naturally in the samples before chloroform addition were not considered harmful to the test objectives. In this test, the DRP levels in some flasks decreased during incubation with chloroform. Consequently, average DRP values from three replicate flasks at each incubation time were compared, and the maximum average DRP level in the sample was selected as the best estimate of the potential available DRP level in the sample. The DRP values in Table 51 indicated the presence of phosphatases, since in most cases the DRP level in the (single) TPP-spiked flask was higher than the mean of the three unspiked flasks. Because of the losses in DRP, especially after about 25 days of incubation, later tests were run for only 7-16 days.

Table 52 lists the maximum DRP values seen in chloroform tests with all river samples, as compiled from Appendix D.* The following mean values and ranges were computed from the data in Table 52, expressed as a percent of TP:

River	Mean	Range
Niagara R. - Ft. Niagara	63	41-91
Niagara R. - Beaver I. Pk.	51	26-80
Genesee R.	38	9-71
Oswego R.	68	52-81
Black R.	45	36-51

It is readily apparent from these numbers that no general trend was seen for TP availability in the Niagara or Genesee River samples, while the Oswego and Black R. samples showed somewhat more consistent behavior in the tests.

Table 51. DRP CHANGES IN CHLOROFORMED RIVER WATERS, WITH AND WITHOUT ADDED SODIUM TRIPOLY PHOSPHATE (TPP)

A. Initial period of storage at 4°C in darkness

Sample No.	Initial DRP[a] (µgP/l)	Storage Period (days)
22-Oswego R. Mouth	58	30
23-Oswego R.	49	30
24-Oswego R. Mouth	41	16
25-Black R.	14	16
26-Oswego R.	79	16

[a] Determined prior to storage period

B. Dark incubation with chloroform at 17-23°C

	DRP[a] after incubation (mean values in parentheses)			
Sample No.	1 day	8 days (µgP/l)	25 days	50 days
No. 22	64	69	75	72
	65 (64)	70 (70)	74 (73)	70 (66)
	64	73	69	55
No. 22 + TPP[a]	77	146	104	101
No. 23	64	72	76	66
	69 (66)	52 (63)	51 (63)	14 (39)
	65	65	63	36
No. 23 + TPP[a]	86	79	87	72
No. 24	59	44	3	12
	59 (59)	52 (46)	40 (22)	58 (36)
	59	41	24	37
No. 24 + TPP[a]	69	90	137	127
No. 25	20	15	16	24
	19 (20)	16 (16)	4 (15)	12 (24)
	19	18	24	37
No. 25 + TPP[a]	33	68	117	100
No. 26	95	46	42	49
	97 (96)	96 (75)	96 (72)	100 (66)
	95	84	79	49
No. 26 + TPP[a]	112	144	152	56

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[a] One ml of 10 mgP/l sodium tripoly phosphate + 100 ml sample water

[b] Samples were filtered through glass fiber filters for DRP analysis

Table 52. MAXIMUM DRP VALUES IN CHLOROFORMED NEW YORK RIVER WATERS AFTER 1 TO 16 DAYS OF DARK INCUBATION

Sample No.	TP (µgP/l)	Maximum DRP observed during incubation[a] (µgP/l)	(% of TP)
		Niagara R. at Ft. Niagara	
27	18	10	56
33	34	14	41
41[d]	22	20	91
50	26	19	73
56	59	31	52
		Niagara R. at Beaver I. Park	
40[d]	15	12	80
49	51	24	47
57	86	22	26
		Genesee R.	
34	386	36	9
42[d]	150	51	34
51	173	122	71
58	204	77	38
		Oswego R.	
22[c] (r. mouth)	93	73	79
23[c]	96	66	69
24[b] (r. mouth)	88	59	67
26[b]	154	96	62
28	93	69	74
29	106	86	81
31	95	49	52
35	105	67	64
43[d]	96	76	79
52	104	72	69
54	87	58	67
55	96	59	62
59	147	91	62
		Black R.	
25[b]	53	24	45
36	34	15	44
44[d]	34	17	50
53	41	21	51
60	99	36	36

[a] Mean values from triplicate flasks

[b] Stored 16 days at 4°C before test

[c] Stored 30 days at 4°C before test

[d] Stored 96 days at 4°C before test

The effect of preliminary storage at 4°C before chloroform addition was studied with one set of samples as shown in Table 53. The Niagara R. sample (No. 40) from the Beaver I. station, along with the Genesee and Black R. samples, showed little or no change in their DRP concentrations during a 96-day cold storage at 4°C. In contrast, the other Niagara R. (No. 41) sample showed an increase of DRP from 18 to 73 percent of TP. Also, the Oswego sample showed an increase of DRP from 40 to 72 percent of TP during the cold storage period. All samples showed increases in DRP as a result of chloroform treatment, but the increases were slight for the Genesee and Oswego R. samples. Substantial percentage changes were seen in the DRP levels of the Niagara and Black R. samples after addition of chloroform and subsequent incubation for only seven days. After seven days the available P was essentially constant or decreased relative to the value at seven days.

Dark Incubation with Resin--

Table 54 shows the results of a dark incubation of a 1:1 mixture of Genesee R. and river mouth water incubated for 100 days, with and without anion-exchange resin. Using the values obtained at 100 days, the calculated DRP from the river water in the two bottles with resin was:

$$76 \ \mu gP/l \times 2 - 35 \ \mu g \ P/l = 111 \ \mu g \ P/l, \text{ or } 70 \text{ percent of TP}$$

The calculated value for the river water in the flask without resin was 116 μgP/l, or 69 percent of TP, which was not considered significantly different from the value with the resin.

The results of other incubations, in which the river water was not diluted by lake water, are given in Table 55 compiled from Tables C.9 to C.13 of Appendix C. The following ranges and mean values were found for the maximum observed mean DRP values in the incubations: (percent of TP)

Table 53. CHANGES IN THE DRP FRACTION OF TP AS A RESULT OF COLD STORAGE FOLLOWED BY DARK INCUBATION WITH CHLOROFORM

	DRP (% of sample TP)				
			Stored 4°C, then chloroformed:[a]		
Sample No.	Initial Value	After 96 days at 4°C in dark	1 day	7 days	14 days
40 (Niagara R. at Beaver I. Pk.)	40	47	67	80	67
41 (Niagara R. at Ft. Niagara)	18	73	82	91	86
42 (Genesee R.)	27	27	29	33	34
43 (Oswego R.)	40	72	76	77	79
44 (Black R.)	26	26	32	47	50

[a] Mean values of triplicate test flasks

Table 54. DRP CHANGES DURING DARK INCUBATION OF EQUAL VOLUMES OF GENESEE R. (NO. 16) AND GENESEE R. MOUTH (NO. 17) WATER SAMPLES

A. Initial Sample Data

	Phosphorus (µgP/l)		
Sample	DRP	TSP	TP
16	43	82	167
17	12	20	43

B. Dark Incubation (Each value represents one test bottle)

	DRP(µgP/l after incubation[a]			
Test Sample	10 days	25 days	50 days	100 days
300 ml No.17 water	32	31	30	34
300 ml No.17 water + 300 ml No.16 water	70	73	75	75
300 ml No.17 water + anion-exch. resin	33	33	39	35
300 ml No.17 water + 300 ml No.16 water + anion-exch. resin	73 69	77 72	77 75	76 75

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[a] DRP in bottles with resin (1g) includes DRP in solution plus DRP on the resin.

Calculations:

1) Bottles with resin
No. 17 DRP at 100 days = 35 µgP/l
No. 17/No. 16 mixture DRP at 100 days = 76 µgP/l
DRP from No. 16 = (2 X 76) - 35 = 117 µgP/l

2) Bottles without resin
No. 17 DRP at 100 days = 34 µgP/l
No. 17/No. 16 mixture DRP at 100 days = 75 µgP/l
DRP from No. 16 = (2 X 75) - 34 = 116 µgP/l

Table 55. MAXIMUM PER CENT OF TP OBSERVED AS DRP DURING DARK INCUBATIONS OF NEW YORK RIVER WATER SAMPLES

Sample No.	TP	Maximum Observed DRP in Incubation [a]			
		Sample	Sample + Resin	Sample	Sample + Resin
	µgP/l	µgP/l		(% of TP)	
		Niagara R. at Ft. Niagara			
33	34	12	8	35	24
41	22	5	4	23	18
50	26	8	5	31	19
56	59	30	26	51	44
		Niagara R. at Beaver I. Park			
32	30	6	4	20	13
49	51	14	7	28	14
57	86	10	9	12	10
		Genesee R.			
16	167	116	117	69	70
34	386	31	63	8	16
42	150	42	39	28	26
51	173	109	110	63	64
58	204	71	77	34	37
		Oswego R.			
23[b]	96	72	76	75	79
24[c] (r. mouth)	88	61	56	69	64
26	154	114	108	74	70
31	95	47	64	50	67
35	105	64	73	61	70
43	96	66	37	69	39
52	104	63	55	61	53
54	87	49	51	56	59
59	147	67	55	46	37
		Black R.			
25[c]	53	29	26	55	49
36	34	10	3	29	9
44	34	6	7	18	21
53	41	7	8	17	20
60	99	24	22	24	22

[a] DRP in flasks with sample + resin includes DRP in solution plus DRP on the resin. All values are mean values from duplicate or triplicate test flasks, except sample no. 16 (see Table 54).

[b] Stored 35 days at 4°C before test.

[c] Stored 21 days at 4°C before test.

River	Mean	Range (no resin)	Mean	Range (resin)
Niagara R. - Ft. Niagara	35	23-51	26	18-44
Niagara R. - Beaver I.	20	12-28	12	10-14
Genesee R.	40	8-69	43	16-70
Oswego R.	62	46-75	60	37-79
Black R.	29	17-55	24	9-49

In some cases, the flasks with resin showed lower values of DRP than did the flasks without resin, so both values were reported in the summary given above. Except in the case of the Niagara River (Beaver I.) samples, the ranges of TP availability were too broad to allow any generalizations to be made.

Autoclave treatment and bioassay--

Table 56 shows the forms of phosphorus found in autoclaved river water samples in comparison to the forms present in the original samples. One significant discrepancy was found between the TSP after autoclaving and the TSP before autoclaving. Sample No. 56 had 33 µgP/l TSP initially and only 17 µgP/l after autoclaving. The DRP dropped from 26 to 6 µgP/l. Smaller TSP losses were seen in Samples No. 27, No. 40, No. 41, No. 28, No. 29, and No. 31. All of these samples were from the Niagara R. or Oswego R. Since the purpose of autoclaving the samples was to estimate the maximum potentially available P in the water, the bioassay values for these samples are probably under estimates of the maximum potentially available P because of possible phosphorus fixation reactions which occurred before the samples were filtered for bioassay.

Table 57 presents the bioassay data which were collected for the purpose of determining whether the TSP measured chemically was actually available for algal growth. The data in this table, based on uncorrected

Table 56. EFFECT OF AUTOCLAVE TREATMENT ON THE SOLUBLE P FORMS IN RIVER WATERS

Sample No.	Initial Soluble P		Soluble P after Autoclaving	
	DRP	TSP	DRP	TSP
	(µgP/l)		(µgP/l)	
Niagara R. at Ft. Niagara				
27	4	8	--	6
33	5	10	--	13
41	4	8	--	6
50	1	7	2	16
56	26	33	6	17
Niagara R. at Beaver I. Park				
32	2	5	--	8
40	6	10	--	6
49	2	6	1	16
57	3	7	5	15
Genesee R.				
34	26	26	--	40
42	40	45	--	44
51	104	111	103	113
58	49	58	52	68
Oswego R.				
28	68	71	--	64
29	78	82	--	76
31	43	49	--	40
35	47	52	--	72
43	38	46	--	53
47	41	50	--	72
52	50	56	52	73
59	46	59	48	76
Black R.				
36	7	12	--	20
53	5	16	18	37
60	13	24	30	51

Table 57. COMPARISON OF SOLUBLE P AND ALGAL-AVAILABLE P IN AUTOCLAVED, FILTERED RIVER WATERS

	P after Autoclaving, Filtering (μgP/l)			
Sample No.	DRP	TSP	Algal-Available P[a]	Bioassay Std. Dev.
Niagara R. at Ft. Niagara				
27	--	6	<2	--
33	--	13	7	1
41	--	6	<2	--
50	2	16	<4	--
56	6	17	9	0
Niagara R. at Beaver I. Park				
32	--	8	<2	--
40	--	6	<2	--
49	1	16	<4	--
57	5	15	4	1
Genesee R.				
34	--	40	42	1
42	--	44	40	3
51	103	113	187	23
58	52	68	81	0.4
Oswego R.				
28	--	64	86	5
29	--	76	124	9
31	--	40	54	3
35	--	72	124	8
43	--	53	54	8
47	--	72	82	4
52[b]	52	73	93	2
52[c]	--	73	103	4
59	48	76	94	3
Black R.				
36	--	20	12	4
53	18	37	23	4
60	30	51	38	3

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[a] Mean values of _Selanastrum_ bioassays, using uncorrected data for quantitation of apparent algal-available P. The bioassay data in this table were derived from the data in Appendix B by multiplying the data in the appendix by 27/20 to account for the sample dilution in the bioassay flasks.

[b] Assayed May 31, 1973.

[c] Assayed June 5, 1973.

A_{750} data indicate that in the Niagara R. and Black R. samples, less P was available to Selenastrum than predicted by the TSP value measured after autoclaving. In the Niagara and Black R. samples whose DRP data was measured after autoclaving, the bioassay values fell between the DRP and TSP concentrations, as expected if there were no gross over or underestimations of the available P. However, apparent overestimations were found in several Oswego and Genesee R. samples where apparent available P was greater than the TSP measured after autoclaving.

As noted in Section VI, the correction of A_{750} data to a cell count basis should account for the optical differences between cells grown in sample water supplemented with AAP(-P) and in AAP(-P) itself. These corrections were made in Table B.29 of Appendix B to produce the data given in Table 58. The following mean values and ranges were calculated from the data:

	Per Cent of TP	
River	Mean	Range
Niagara-Ft. Niagara	<12	9-15
Niagara-Beaver I. Pk.	< 8	5-<13
Genesee R.	32	8-72
Oswego R.	60	38-84
Black R.	43	35-56

The values from the Black R. samples were not corrected values, since none of the Black R. samples had been used to construct the correlation curve shown in Figure 12. Only the Niagara and Black R. samples showed relatively consistent bioassay results, in terms of the per cent of TP available after autoclaving and filtering. The ranges for the other rivers were very broad.

Table 58. COMPARISON OF TSP AND ALGAL-AVAILABLE P, CALCULATED USING CORRECTED A_{750} DATA IN AUTOCLAVED, FILTERED RIVER WATERS

Sample No.	P (µgP/l) TSP	P (µgP/l) Algal-Available P[a]	P (% of TP) TSP	P (% of TP) Algal-Available P
		Niagara R. at Ft. Niagara		
27	6	<2	33	<11
33	13	5	38	15
41	6	<2	27	< 9
50	16	<4	62	<15
56	17	7	29	12
		Niagara R. at Beaver I. Park		
32	8	<2	27	< 7
40	6	<2	40	<13
49	16	<4	31	< 8
57	15	4	17	5
		Genesee R.		
34	40	30	10	8
42	44	31	29	21
51	113	124	65	72
58	68	57	33	28
		Oswego R.		
28	64	61	69	66
29	76	88	72	83
31	40	36	42	38
35	72	88	68	84
43	53	39	55	41
47	72	57	74	58
52[b]	73	63	70	60
52[c]	73	72	70	69
59	76	65	42	44
		Black R.[d]		
36	20	12	59	35
53	37	23	90	56
60	51	38	52	38

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[a] Mean values of Selenastrum bioassays, using A_{750} data corrected as explained in the text. The corrected A_{750} data are given in Table B.29 of Appendix B , along with the apparent available P values in the assay flasks. The assay flasks values were multiplied by 27/20 (to correct for sample dilution in the bioassay flask) to obtain the data given in this table.

[b] Assayed May 31, 1973

[c] Assayed June 5, 1973

[d] Black R. samples were not quantitated with corrected data; the values given in this table are taken from Table 57 data.

SECTION VIII

DISCUSSION

MADISON URBAN RUNOFF

Stormwater samples collected in Madison, Wisconsin, from August 1972 to March 1973 showed a wide range of total phosphorus concentrations, from 59 μgP/l (see Table 31). These concentrations were probably the result of the following factors: (1) the different street solids loading intensities expected for different urban land usages (APWA, 1969; Sartor and Boyd, 1972), (2) the changes in TP concentration with time during the runoff event (Kluesener, 1971; Weibel *et al*., 1964), and (3) the additional phosphorus inputs from autumn leaf-fall (Cowen and Lee, 1973) or from construction activities (Ryden *et al*., 1972), which modify the normal solids loading intensity for a given urban land use. Regardless of the absolute values of TP in the samples, however, the soluble and particulate P forms in the samples were expected to qualitatively represent the nature of the materials transported to the Madison lakes in urban runoff.

Particulate P Availability

Mean values of acid and base extractable inorganic P (PP_i) from PP contained in urban runoff ranged from 8 to 60 per cent of PP, with considerable overlap of the values for the two extraction methods (see Figure 15). The overlap was expected, since the acid extraction should dissolve calcium phosphate completely and aluminum and iron bound phosphate considerably, while the base extraction should dissolve aluminum and iron bound phosphate, but not calcium phosphate (Chang and Jackson, 1957). Thus, especially if the content of calcium phosphates in the particles was low, the two extraction procedures could yield similar results. The degree of hydrolysis of organic phosphates with these procedures was not evaluated but could include both hydrolysis during the extraction and in the acidic color reagent used in the Murphy and Riley (1962) colorimetric test for DRP. The latter step was considered by Chamberlain and Shapiro (1973) to cause litte hydrolysis of glucose-1-phosphate, which is reported to be a very labile ester (Weil-Malherbe and Green, 1951). Consequently, hydrolysis was likely minimal in the DRP analysis procedure. However, since the filtered extracts were often stored (4°C) overnight before the analysis for inorganic P, and because of the long duration of the base extraction (overnight), some hydrolysis of organic esters may have occurred in the extraction medium.

The results shown in Table 32 and Figure 15 indicated that the range of mean values for acid extractable PP_i in residential runoff samples was greater than the range of mean values for base extractable PP_i in the same samples. The greater variability between samples of the acid extractable fraction may have been due to differences in the relative amounts of calcium-bound P in the particulate matter, since calcium-bound P would be extracted by the acidic treatment but not by extraction with base. Further, the acid extractable PP_i likely approaches the total inorganic P content, as is the case for many lake sediments (Williams _et_ _al_., 1971b).

When the group mean values from the various urban sampling sites were compared for a given extraction method, there appeared to be very little difference between the various land uses sampled. As shown in Figure 15, all group mean values for acid extractable PP_i fell within the range of 33 to 46 per cent of PP, while all group mean values for base extractable PP_i fell into the range of 22 to 27 per cent of PP. Possibly, the dominant form of PP in all samples was derived from a common source input, such as dustfall or eroded soil. Sartor and Boyd (1972), in a study which included industrial land uses, noted that while the various types of urban areas exhibited different loading intensities of total P in dry street surface contaminants (industrial > residential > commercial), the PO_4 content of the solids did not appear to differ appreciably among the various land use types (0.103 to 0.142 per cent by weight).

In contrast to the treatment of runoff PP with acid and base, which dissolved certain phosphatic components of the PP, the resin extraction was a relatively mild treatment, designed to measure only the inorganic P which was involved in exchange reactions between the solid and solution phases. Thus, the resin method was expected to produce lower values of extractable PP_i than the acid or base methods. This was actually the case, as demonstrated by the group mean values shown in Figure 15. The range of mean values for sample replicates was 2 to 28 per cent of PP (Table 32), but the group mean values for the various land uses were in the range of 13 to 17 per cent of PP.

Long-term (26 days) dark incubations of particles from runoff samples D-11, E-11, and H-11 in unfiltered lake waters containing anion-exchange resin showed resin extractable PP_i values of 21, 8, and 10 percent of PP, respectively (Tables 34 and 35). In comparison, the short-term (24 hour) resin extraction of these particles in distilled water gave extractable PP_i values of 20, 16, and 20 percent of PP, respectively (Table 32). Since the DRP levels in both systems were maintained low by the resin, the major differences between the systems were the presence of lake water organisms and chemical constituents, and the time allowed for equilibration. Considering the errors inherent in the lake water experiment due to subtraction of control values from test values, the two procedures yielded similar results. This would indicate that physical-chemical sorption-desorption and precipitation-dissolution reactions were more important in controlling the release of inorganic P than were biological processes, such as DRP release from autolysis of microbial cells in the PP. The increases in DRP observed in lake water control flasks (no runoff particles added) between 1 and 13 days demonstrated mineralization of detrital phosphorus to DRP, as reported in several investigations involving dark incubations of sea water (Waksman et al., 1937; Renn, 1937).

Long-term dark incubations of runoff particles in lake waters without anion-exchange resin demonstrated DRP removal from the lake water by sample E-11 and H-11 particles, while the particles from sample D-11 showed essentially no net contribution or removal of DRP. As discussed by Taylor and Kunishi (1971) and Ryden et al. (1972), particles of eroded soil have the potential to gain or lose inorganic P in the receiving water, depending upon the nature of the soil P and upon the DRP level of the receiving water. Because of the dependence upon the

DRP level in the receiving water, any results obtained from tests with resin would be applicable only to receiving waters with low DRP levels, such as the epilimnion waters of the Madison lakes in the late summer. Also, since the resin uptake might simulate DRP uptake by algae, the resin tests might be representative of low DRP waters which exhibit a high algal demand for P.

The addition of orthophosphate spikes to unfiltered runoff sample D-11 demonstrated that the particles in the runoff were capable of rapid fixation of inorganic P (Table 36). Resin extraction of the spiked runoff sample replicates showed that all of the added spike was recovered from the solution phase and the solid phase of the runoff. Since these tests were performed in a total elapsed time of just 48 hours, biological release of DRP from autolysis of organisms was considered unimportant, although uptake of the orthophosphate spikes by microorganisms could have occurred (Phillips, 1964). However, the recovery of phosphate which had been fixed by the particles indicated that sorption-desorption processes were probably more important than biological reactions. The slight excess (6 to 11 percent) recovered P over 100 percent from the resin extraction was likely derived from exchangeable P originally associated with the PP in the runoff.

In contrast to the short-term (24 hour) direct extractions of PP with resin (Table 32), long-term (up to 50 days) dark incubation of unfiltered runoff (Table 37) was designed to allow biological reactions to occur in the runoff particles. As indicated in Table 59, the estimated inorganic P released from PP (Maximum R - DRP - Initial sample TSP) in the long-term incubations were significantly (10 or more percent) larger than the short-term direct

Table 59. COMPARISON OF RESIN EXTRACTABLE PP_i FOR DIRECT SHORT-TERM EXTRACTION OF PP AND FOR LONG-TERM DARK INCUBATIONS OF UNFILTERED RUNOFF

	Resin Extractable PP_i (% of PP)	
Sample No.	Direct short-term Extraction of PP[a]	Calculated[b] from long-term dark incubations of unfiltered runoff
A-8	22	20
A-9	15	-4
A-12	18	17
B-4	19	32
B-7	12	44
B-8	19	12
B-9	15	-8
B-12	17	9
D-8	15	20
D-10	12	15
D-11	20	13
D-12	20	14
E-11	16	1
H-11	20	-4
F-7	21	31
F-8	16	2
I-12	13	7

[a] Mean values, from Table 32

[b] Calculated using equation (1) in text and data from Table 37

extraction values in only 3 of the 17 samples tested (samples B-4, B-7, and F-7). In five samples, the results of the long-term dark incubations were significantly lower than the results of the short-term PP extractions. Both test systems contained anion-exchange resin, to promote desorption of inorganic P from the PP forms. However, in the long-term dark incubations of unfiltered runoff the soluble chemical constituents of the runoff were present along with the sample PP and resin. In the direct resin extraction of PP over 24 hours, only the resin and sample PP were present in the distilled water medium. Possibly the soluble chemical constituents of the runoff may have competed for the available sorption sites on the resin, thus reducing the capacity of the resin for inorganic P adsorption. Such competition was absent in the distilled water-resin extraction system.

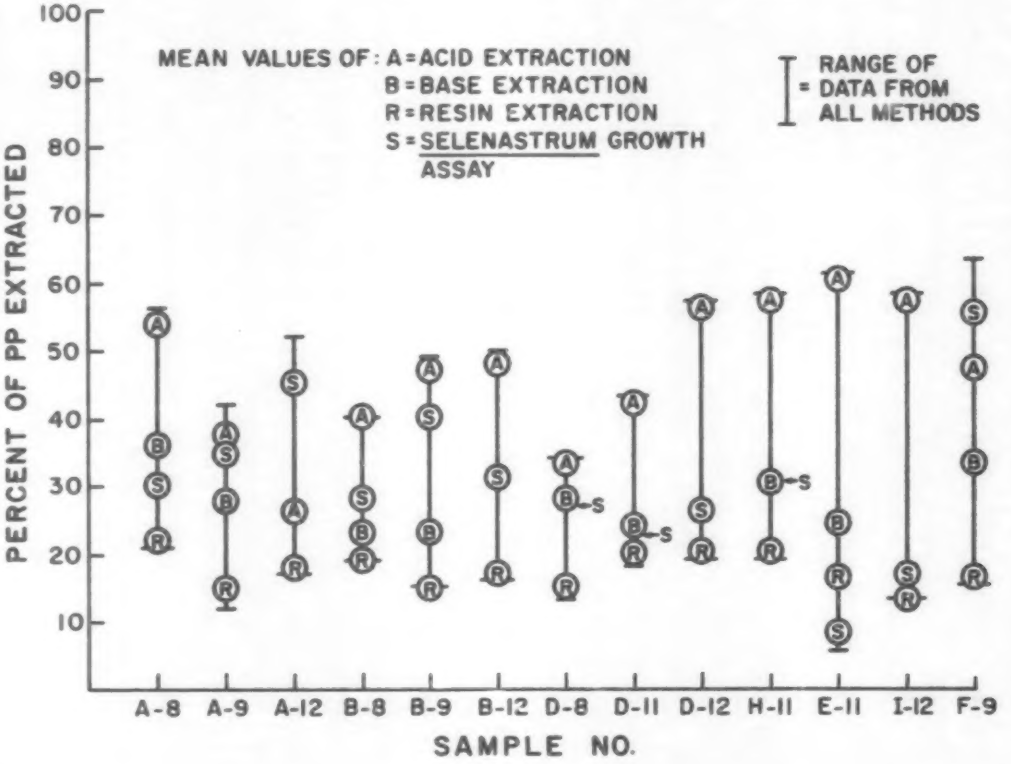
Although the unfiltered runoff samples were incubated for long periods, no significant release of DRP was detected in most samples, either from desorption or mineralization reactions, such as hydrolysis of organic P or bacterial release of inorganic P as a result of death and cell lysis. Nine of the samples showed values which were in agreement (to within 10 percent of PP) for the long-term and short-term test systems. Since the short-term system evaluated only the physical-chemical reactions of the PP, while the long-term system evaluated both physical-chemical and biological reactions of the PP, agreement of the two test systems indicates that physical-chemical processes are the key factors controlling the release of inorganic P to solution from particulate P forms. Only in the three samples (B-4, B-7, and F-7) where the results of long-term incubations significantly exceeded the results of the short-term tests was there evidence for significant inorganic P release from biological reactions in the runoff particles.

Direct biological assessment of the algal-available fraction of PP was sought by growing Selenastrum in a P-free algal medium with the runoff particles as the sole source of P to the algae (Table 33). An overall range of 8 to 55 percent of PP was found for the 13 samples tested, mostly from residential sites. The mean value for all samples was 30 percent, a value which was intermediate between the ranges of group mean values from the acid and base extractions of PP, 33 to 46 and 22 to 27 percent of PP, respectively. However, such comparisons are not strictly valid, since some of the samples were not analyzed by all three methods. Figure 18 compares the results of the various PP extraction methods and the algal bioassay on a sample-by-sample basis. The ranges shown represent the range of the individual replicate determinations from all methods on a given sample.

With three exceptions, the bioassay results fell within a range established by the mean values of the acid and resin extractions. In six of the 10 samples tested by bioassay and base extractions, the results agreed to within 10 percent (of PP) or less. In contrast, the bioassay and acid extraction results were within 10 percent in only 4 of 13 samples. The studies of Golterman _et al_. (1969) and Fitzgerald (1970b) have indicated that some relatively insoluble iron and calcium phosphates could be used for growth by _Selenastrum_, _Chlorella_, and _Scenedesmus_ cells. Thus, some agreement between the results of bioassays of runoff PP and extractions of PP which dissolve iron and calcium phosphates might have been expected from the reports in the literature. The resin extraction data was in essential agreement with bioassay results (10 percent or less) for 7 of the 13 samples tested, so apparently the resin and base extraction methods were measuring "available" PP more often than was the acid extraction method.

The test most likely to predict the true availability of PP forms in the receiving water cannot be definitely determined from the data reported here. The factors which will affect the eventual contribution of inorganic

Figure 18. Comparison of chemical and biological extraction of PP (Madison urban runoff samples)



P from PP will include, besides the chemical forms of PP in the runoff, the temperature and DRP concentration of the receiving water, the steepness of the lake basin at the point of discharge, the rate of sediment deposition and mixing in the area of deposition, and finally, the density and diameter of the particles themselves.

Large, high density particles, such as those observed in samples of runoff from both residential and urban construction areas, would rapidly settle out of the water column in the absence of turbulence at the point of entry into the lake. If the littoral zone of the lake is not steep at this point, the particles will remain in the photic zone of the lake, where they could release phosphorus to the water for phytoplankton growth, or to rooted aquatic plants. Martin et al. (1969) have shown that lake muds are the principal source of nutrients for the (rooted) aquatic weed, Najas. The temperature and DRP level of the lake water, as well as the degree of sediment-water mixing would also affect the fraction of PP contributed to the lake water as available P. Cooke and Hislop (1963) reported that the degree of exchange of phosphorus from coils to anion-exchange resin was temperature dependent, with a two-fold increase in phosphorus extractability between 10 and 30°C. A temperature of 15-22°C was used in the studies with Madison runoff and Lake Ontario tributaries, to simulate natural water temperatures. The DRP level of the receiving water would affect the sorption or desorption of inorganic P from soil particles, as predicted from the phosphate sorption curves of Ryden et al. (1972), which related the concentration of inorganic P in the aqueous phase to the change in concentration of inorganic P on the particles in equilibrium with the aqueous phase. Low

DRP levels in the receiving water would increase the potential desorption of inorganic P. However, the reverse (sorption) at high DRP levels in the water may not necessarily hold for some eroded soils. Ryden et al. (1972) found that soil from an Al horizon was capable of releasing an appreciable amount of inorganic P to solution, even in the presence of relatively high levels of added inorganic P. A dissolution reaction of a slightly soluble soil-fertilizer P reaction product in this horizon was suggested as the reason for the P release characteristics of the Al horizon. If temperature and DRP concentration are suitable for P release, the actual release may still be limited by the degree of mixing of the particles with the water column. Also, further sedimentation would hinder release by covering previously deposited material, as in the foreset beds of a delta.

Small, low density particles would remain in suspension in the photic zone for relatively long periods of time compared to large, high density particles. Therefore, the small particles should be more important to the chemical and biological availability predicted in the tests discussed above. Particles remaining in suspension would be in close contact with planktonic algae, as in the algal bioassay, and if the soluble inorganic P level of the receiving water were very low, algal uptake might approximate the phosphorus availability predicted by the resin extractions of PP forms in distilled water. Evidence from other studies indicates that much of the PP in runoff is associated with fine particles. Sartor and Boyd (1972) reported that up to 55 percent of the P by weight in dry street contaminants was associated with particles less than 43 microns in diameter. The loadings of phosphorus from dustfall reported by Kluesener (1971) and Johnson *et al*. (1965) of 0.7 lbs. total P/acre/year and 0 to 0.45 lbs. water-soluble P/acre/year, respectively, are indications of the potential contribution of small particles to total runoff phosphorus. Much of the fine particulate matter, falling on impervious surfaces, is readily transported by surface runoff, which has been estimated to carry an annual load of 1 lb. total P/acre from all source inputs of phosphorus in the urban environment (Kluesener, 1971). Thus, the urban loading of dustfall P and yield of runoff total P are seen to be different by perhaps no more than a factor of two or less (0.45 to 0.7 compared to 1.0). Evans *et al*. (1968) were only able to reduce the TP content of runoff samples by 30 percent in 5 hours of settling time in the laboratory, although the suspended solids concentration was reduced by 70 percent in the tests.

Because of all the considerations discussed above, the results of the chemical and biological tests of PP availability are probably overestimates of the true PP availability in the receiving water. However, the bioassay and short-term direct resin extractions of PP are probably the best estimators of the true behavior of the particles, based on theoretical considerations. The mean values of PP availability determined by these two tests have been summarized for all samples tested, as follows:

	Mean Percent of PP "Available"	
Test	Range	Ave. of Mean Values
Resin Extraction	2-28	15
Selenastrum Bioassay	8-55	30

Soluble P Availability

The bioassays of membrane-filtered runoff showed three classes of response (Table 38): (1) available P greater than 114 percent of TSP, (2) available P between 98 and 114 percent of TSP, and (3) available P less than 60 percent of TSP. In membrane-filtered samples, the TSP value represented the maximum possible concentration of available P, since it was considered improbable that any phosphorus available for algal growth would not be measured by the persulfate digestion method for TSP. Samples whose available P levels fell within 98 to 114 percent of TSP (A-8, D-8, D-10, and D-11) apparently contained soluble P compounds whose biological availability roughly matched their liability to persulfate digestion.

Samples whose available P was less than TSP (H-10, H-11, E-10, E-11, and I-12) apparently contained dissolved P forms which were measured by the chemical digestion procedure, but were not available for algal growth. Since the available P levels in all these samples were less than even the DRP levels, the chemical method for DRP may have overestimated the available P in these filtrates. Alternatively, the bioassay may have been in error, due to the difficulty in establishing a standard curve for the bioassay of soluble P as discussed below. The DRP in the filtrates may have included some colloidal inorganic P, which could pass the filter and be dissolved by the acid conditions of the DRP analysis and the very acidic conditions of the persulfate digestion for TSP. The biological availability of such forms would likely be less than that of dissolved orthophosphate-phosphorus. Chamberlain (1968) found that approximately 90 percent of the ^{32}P in membrane-filtered (0.45 micron pore size) lake waters, prepared with ^{32}P before filtration, could be removed by refiltering the lake water through a 0.01 micron pore size membrane filter. In a parallel test, 80 percent of the ^{32}P could be sedimented from the membrane filtered lake water by ultracentrifugation. Other possible sources of nonavailable "soluble" P include condensed or organic P compounds which are hydrolyzed in the chemical analyses but are not available to the test algae in the bioassay. Chamberlain and Shapiro (1969) have pointed out that arsenate may also appear as "available P" (phosphate) in the chemical test for DRP. None of these sources of error can be ruled out until more data is collected on the soluble P forms in urban runoff.

Six of the runoff samples tested showed algal-available P higher than 114 percent of TSP (Sample B-9 is included in this group, with available P reported as greater than 110 percent of TSP). Since this was theoretically impossible, an error in the bioassay procedure was indicated. In all the bioassays of runoff soluble P, the filtrates were supplemented with concentrated AAP (-P) medium such that in the assay flasks there would be at least the same concentration of AAP (-P) nutrients as were present in the AAP standard flasks. However, unlike the standard AAP cultures, the sample cultures also contained the nutrient salts from the original runoff, diluted somewhat by the added AAP (-P) spike and algal inoculum. Thus, even though phosphate limitation existed in both the sample and standard cultures, the nutrient balance differed and produced differences in the optical properties of the cells. In Figure 16, the curve of A_{750} versus added orthophosphate spikes for sample B-9 was transposed to begin at 167 µgP/l for the unspiked sample, since 167 µgP/l was the TSP concentration in this sample culture. It could have been placed at any point less than the TSP value; the same conclusion would have been reached, namely that the true response curve represented by the extrapolation of the spiked sample response curve back to 0.0 μgP/l was to the left of the standard response curve. Thus, at any given A_{750} value for a sample culture, the standard curve would overestimate the available P concentration in the sample culture flask.

Because of the problems involved with high values of apparently available TSP, definite conclusions about the relative availability of the TSP forms in Madison urban runoff could not be made. However, the low results from some of the samples do indicate that the chemical analyses of soluble P forms should not be accepted as accurate measurements of available P unless they have been correlated with biological tests.

PRECIPITATION SAMPLES

Madison Snow Samples; PP Availability

Chemical analyses of Madison snow samples for phosphorus forms showed that most of the phosphorus was in a particulate form, probably from dustfall on the snow or crystallization of the snow around atmospheric dust particles. The biological availability of the snow PP ranged from less than 2 to 23 percent of PP (Table 39). The dustfall samples collected by Kluesener (1971) showed about 14 percent of the total P in the dustfall to be leachable as DRP. Since by definition "dry fallout" or dustfall is entirely particulate in nature, Kluesener was actually measuring the percent of dustfall PP which could be leached as DRP. Consequently, his data are comparable to the bioassay results reported here.

The snow sample which showed less than 2 percent of PP to be algal-available was collected in a commercial section of Madison (the roof of the City-County building), where the automobile traffic is very heavy. Sartor and Boyd (1972) reported that the concentrations of zinc, copper, and lead were highest in street surface contaminants from commercial areas, as compared to materials from industrial or residential areas. Thus, the possibility of toxicity in the bioassay of the commercial area PP must be considered as an explanation of the apparent low P availability in this sample. These results are only intended as preliminary estimates of the PP availability of dustfall. Unfortunately, more samples of this type were not collected during the winter of 1972-1973.

New York Rain Gage Samples; TP Availability

These samples displayed a unique mixture of phosphorus forms, as demonstrated by the data in Table 40. Several samples showed very high TSP levels along with relatively low DRP concentrations. Such data indicated that the filtration procedure may have included phosphorus forms which were dissolved but not reactive, or which were not truly dissolved species in the filtrates analyzed for DRP and TSP. Generally, in runoff waters, the concentrations of DRP and TSP were of comparable magnitude (see Table 31). The presence of colloidal, acid soluble phosphorus forms would not in itself explain the differences seen, unless the colloidal forms required oxidation for release of their phosphate to solution as inorganic P.

The bioassay of unfiltered rain gage waters with "abnormal" TSP concentrations showed that the algal-available P levels were comparable to the DRP concentrations, and hence much lower than the TSP (or TP) levels.

Table 60 compares the chemical and biological analyses of the rain gage samples. If the samples with extremely low TP values are disregarded, only two samples, June 601 and June 604, contained algal-available P concentrations of the same order of magnitude of DRP and TSP. Since only two sets of rain gage samples were received from New York State, any conclusions relating TP availability (range: <1 to 90 percent of TP) to the location or condition of the gage (open or closed) would be extremely difficult. The results of this short study serve to point out that care must be taken in the interpretation of chemical analyses of the phosphorus forms, especially TSP, in such samples.

GENESEE RIVER BASIN SAMPLES; PP AVAILABILITY

Land use in the Genesee R. basin study varied from pasture land to high density residential area. The latter was of interest in comparison to the Madison urban runoff study. Samples from the non-urban land use areas in the Genesee R. basin were frequently so dilute that direct extractions of the PP in these samples were difficult. Consequently, most of the estimates of PP availability were made on samples from the residential areas, Stations No. 2 (Rochester East), and No. 7 (Dansville).

The group mean values of acid extractable PP_i from Stations No. 2 and No. 7 were 48 and 30 percent of PP respectively. These values were just outside the range of the group mean values from the Madison urban runoff PP, 33 to 46 percent, although the individual sample values showed ranges which overlapped (Figures 15 and 17).

Table 60. COMPARISON OF CHEMICALLY AND BIOLOGICALLY DETERMINED PHOSPHORUS FORMS IN NEW YORK RAIN GAGE SAMPLES

Sample No.	DRP	TSP	TP	Algal-Available P[a]
		μgP/l		
May 601-0	60	245	420	42
May 602-C	8	62	86	4
May 603-C	0	2	4	<14
May 604-C	0	2	6	<14
May 605-C	0	304	350	<14
May 606-C	1	401	439	<3
June 601-C	52	63	72	42
June 602-C	4	6	10	<14
June 603-0	5	82	106	4
June 604-C	48	58	64	58
June 605-C	0	6	9	<14
June 606-C	1	6	12	<14
June 608-0	1	318	346	<14

[a] Mean values, from Table 41

The group mean values of the base extractable PP_i from Stations No. 2 and No. 7 were 30 and 18 percent of PP, respectively. These values were lower than the acid extraction values for the same samples and just outside the range of the group mean values from base extractable PP_i in the Madison samples, 22 to 27 percent. Comparison of Figures 15 and 17 shows that there was an overlap in the ranges of the base extraction data from the Madison and Genesee basin samples.

The resin extractable PP_i data from the Genesee R. basin Stations No. 2 and No. 7 showed group mean values of 25 and 11 percent of PP, respectively. In comparison, the group mean values from the Madison samples fell into the range of 13 to 17 percent. As with acid and base extraction data, however, there was considerable overlap between the ranges of the resin data shown in Figures 15 and 17.

Like the PP in Madison samples, the PP from Stations No. 2 and No. 7 of the Genesee R. basin had group mean values of extractable PP_i in the order of acid > base > resin. However, unlike the Madison PP samples, whose group mean values for a given extraction method all agreed to within 13 percent (of PP) or less, the acid extraction group means for Stations No. 2 and No. 7 differed by 18 percent. The differences between the group mean values of the base and resin extractions of PP from Stations No. 2 and No. 7 were 12 and 7 percent, respectively. In all methods of PP extraction, the Station No. 2 materials showed the highest values of extractable PP_i.

The results of the extractions of PP forms contained in runoff from Stations No. 1, No. 4, and No. 9 were close to the results of extractions of PP from Station No. 7 samples, even though the runoff from the former stations was derived from cropland (No. 1), pasture (No. 4), and brushland (No. 9).

Long-term dark incubations of unfiltered runoff Samples No. 402-8, No. 407-8, and No. 507-13 with resin yielded the following estimated inorganic P released from PP (R-DRP-Initial sample TSP), as compared to the results of short-term (24 hr.) direct extractions of PP with resin (see Table 43):

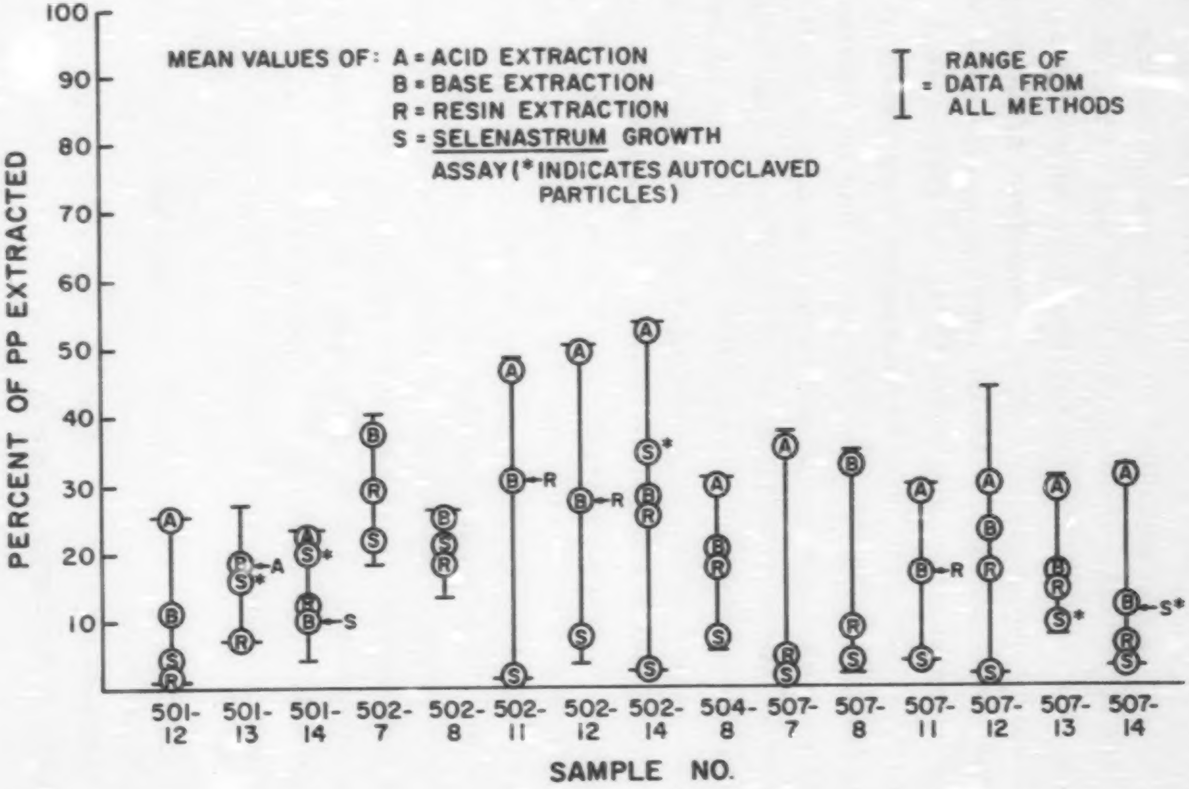
	Percent of PP Extracted by Resin	
Sample No.	Long-term Dark Incubation of Runoff	Short-term Direct PP Extraction
402-8 (Rochester East)	28	25
407-8 (Dansville)	22	11
507-13 (Dansville)	7	13

The long-term incubation (50 days) values were close to the values from the short-term resin extractions of PP in distilled water, except in the case of Sample No. 407-8. The larger values from the long-term incubation of this sample may have been the result of mineralization processes occurring in the particles during the dark incubation.

The direct bioassay of PP by Selenastrum is compared to the other extractions of PP in Figure 19. In all cases where both a resin extraction (short-term, in distilled water) and a Selenastrum bioassay were run on the PP, the bioassay value (S) was essentially equal to or less than the resin extraction value. Since in most cases, the base extraction values were close to the resin extraction values, the bioassay values agreed nearly as well with the base extraction as with the resin extraction values. For simplification, bioassay values listed as "less than" a given cutoff P concentration (Table 44) were plotted at the cutoff value in Figure 19. Acid extraction values were generally significantly higher than the bioassay values, which ranged from <1 to 24 percent of PP. The acid extraction range was, in contrast, 18 to 60 percent of PP.

Bioassays on particles which had been autoclaved in their AAP (-P) medium suspensions showed a slightly

Figure 19. Comparison of chemical and biological extraction of PP
(Genesee R. basin samples)



higher availability range (8 to 34 percent of PP) than did "natural" particles. In one of the samples tested by both bioassay procedures, (No. 507-14), the increase in available PP due to autoclaving was only 7 percent of PP. Figure 19 shows that even after autoclaving, the new bioassay value of 10 percent(S*) was not much different from the resin extraction, base extraction, or original bioassay value. It appears that biological phosphate was not released in large quantity as a result of the autoclaving, or the particles resorbed most of the inorganic P which should have been released from lysed microbial cells in the PP. Physical-chemical phosphorus fixation mechanisms would seem to be dominant in such samples as those from Station No. 7 (Dansville- high density residential). Sample No. 507-13 also showed a bioassay value after autoclaving which was close to the resin and base extraction values. Samples of PP from other stations (No. 501-13, No. 501-14, and No. 502-14), in contrast, tended to show bioassay values after autoclaving which were higher than the resin extraction values or the bioassay values for "natural" particles.

In summary, the expected percentage of available PP in the Genesee R. basin samples (as estimated by the short-term direct resin extraction, base extraction, and bioassay (unautoclaved) mean values from all samples tested) would be:

	Mean Percent of PP "Available"	
Test	Range	Average of mean values
Resin Extraction	1-30	16
Base Extraction	10-37	22
Selenastrum Bioassay	<1-24	<9

NEW YORK RIVER SAMPLES

Particulate P Availability

Direct chemical extraction of PP forms isolated from river water samples was attempted only for Genesee R. samples. Table 47 demonstrated that the chemical nature of the PP forms in this river was quite variable in the different samples obtained during the spring of 1973. The short-term direct resin extraction data for Genesee R. Sample No. 42 showed 23 percent of PP extracted, which compared fairly closely to the results of the long-term dark incubation of Sample No. 42 particles in Lake Ontario water containing anion-exchange resin (18 percent of PP; Table 48).

Selenastrum growth assays of the PP from Genesee R. samples showed that less than 6 percent of the PP was available to *Selenastrum*. The apparent contradiction between these data and the short-term resin extraction data, which showed 6 to 31 percent of PP resin extractable, may be explained by available P uptake by competing organisms native to the water samples, in the algal growth assays. Several species of diatoms were observed during the microscopic counting of *Selenastrum* cells in the 18-day growth assays. Although the short-term resin extractions were also performed in a lighted room, the 24 hr. resin equilibration period was likely too short for significant growth of natural algae, compared to the 18-day algal assays, so that very little competition for dissolved inorganic P was expected from natural algae. Also, the resin extractions were performed in a distilled water medium, which was not as conducive to algal growth as was the AAP (-P) algal assay medium.

The competition for DRP was eliminated in the algal assays by autoclaving the suspensions of PP in AAP (-P) medium, before inoculating with the test organism. Autoclaved particles from two Genesee R. samples, No.58 and No.51, showed 36 and 41 percent of PP available, respectively, compared to 6 and 31 percent for short-term, resin extractable PP_i. The higher values for autoclaved PP bioassays compared to short-term resin extractions may be partially due to release of cellular phosphates from native microorganisms lysed during the autoclaving procedure. These phosphates would not be extractable by the anion-exchange resin. Alternatively, some of the P forms in the autoclaved suspensions may have been available to algae via phosphatase enzyme hydrolysis, yet not measured as DRP in the resin extraction procedure.

Samples of PP from the Niagara, Oswego, and Black Rivers also showed less than 6 percent of the PP to be available to Selenastrum, unless the PP suspensions were autoclaved prior to bioassay. Autoclaved suspensions of PP from these rivers showed 26 to 57 percent of PP available to Selenastrum. Because of the lysis of cellular biomass in the autoclaving procedure, the results of such assays should be regarded as indications of the long-term availability of the PP forms in Lake Ontario.

The bioassays of "natural" particles demonstrated that the particles themselves were not inert, but were capable of holding available P during incubations under light. Much phosphorus may have been "biologically available" from the PP during these assays, but it could not be measured by looking only at the growth of the inoculated test organism. The short-term resin extraction procedure attempted to measure only the organic P bound to the PP by physical-chemical forces, hence that P which would be released during periods of low DRP levels in Lake Ontario. By not allowing the released P to be taken up by a growing population of native algae, as in the bioassay of "natural" particles, the resin procedure probably gave a better estimate of the readily available P than did the bioassay of "natural" PP.

Several treatments of unfiltered river water were performed in an effort to gain some insight into the long-term availability of the total phosphorus in the samples. By using equation (1), below, the contribution of DRP from PP in the samples was estimated:

$$(1)\quad \begin{array}{l}\text{Maximum observed}\\ \text{Algal-available P}\\ \text{or DRP}\end{array} - \begin{array}{l}\text{Initial value}\\ \text{of sample TSP}\end{array} = \begin{array}{l}\text{Estimated con-}\\ \text{tribution of DRP}\\ \text{from sample PP}\end{array}$$

It should be noted that this equation would underestimate the contribution of algal-available P or DRP from PP in those samples where the initial dissolved unreactive P concentration (TSP - DRP) was high and was not used for algal growth or converted to DRP during the test. In those cases, DRP from the particles equal to any unavailable dissolved unreactive P would not be counted by equation (1) in the estimation of available P from PP. However, *at least* the P computed from (1) had to come from PP. The contributions calculated by equation (1) were expressed as a percentage of sample PP, in order to compare the calculated values with the results of direct bioassays of PP.

Table 61 lists the calculated PP availability figures, from (a) chloroform treatment of river waters, (b) long-term dark incubation of river waters with and without anion-exchange resin, where the values reported in Table 8.3 was computed using either the value with or without resin in equation (1), whichever value was larger, and (c) AAP bioassays of autoclaved, filtered river waters, using corrected A_{750} data from Table 58.

Table 61. ESTIMATED PERCENT OF PP AVAILABLE, AS CALCULATED[a] FROM TREATMENTS OF UNFILTERED RIVER WATER SAMPLES

Sample No.	Percent of PP Available as: DRP after chloroforming[b]	DRP or R-DRP after dark incubation[c]	Algal-available P in AAP bioassay[d]
		Niagara R. at Ft. Niagara	
27	20	--	0
33	17	8	0
41	86	0	0
50	63	5	0
56	0	0	0
		Niagara R. at Beaver I. Park	
32	--	4	0
40	40	--	0
49	40	18	0
57	19	38	0
		Genesee R.	
16	--	41	--
34	3	10	1
42	6	0	0
51	18	0	21
58	13	13	0
		Oswego R.	
23	21	47	--
26	13	40	--
28	0	--	0
29	17	--	25

(continued)

Table 61. ESTIMATED PERCENT OF PP AVAILABLE, AS CALCULATED[a] FROM TREATMENTS OF UNFILTERED RIVER WATER SAMPLES

	Percent of PP Available as:		
Sample No.	DRP after chloroforming[b]	DRP or R-DRP after dark incubation[c]	Algal-available P in AAP bioassay[d]
31	0	28	0
35	28	40	68
43	60	40	0
47	--	--	15
52	33	15	15-33
54	20	0	--
55	28	--	--
59	36	9	7
		Black R.	
25	15	29	--
36	14	0	0
44	10	0	--
53	20	0	28
60	16	0	19

[a] Calculated from the maximum mean observed values from the various treatments, using equation (1) of the text.

[b] Calculated from data in Table 52.

[c] Value given is the larger of the two values found with and without anion exchange resin added to the samples; calculated w/Table 55 data.

[d] Calculated with data from Table 58.

The value of 60 percent of PP available was net or exceeded only in four samples. However, it was difficult to draw any conclusions about these results because of the variability between samples from a given river. Only the chloroform-treated Genesee R. and Black R. samples appeared to show consistent results.

The PP availability calculated from the chloroform treatment of Genesee R. Samples No.51 and No.58 was 18 and 13 percent, respectively, compared to values of 41 and 36 percent, respectively, for direct bioassays of autoclaved PP (Table 49) . Sorption of some of the phosphorus released by chloroform-induced lysis may have been responsible for the low values from chloroform treatment of these samples. The dark incubations of unfiltered Genesee R. samples also showed very low values of calculated DRP from PP, even though most of the values in Table 61 were from test flasks, containing anion-exchange resin (except Sample No.42, where the incubation without resin produced slightly higher results). The resin apparently had a negligible effect on the long-term indications, however. Sorption by the suspended soils may have been effective in competing with the resin for phosphate. In the single test of Sample No.16 Genesee River water (300 ml) mixed with Lake Ontario water (300 ml), relatively high proportion (40 percent) of the PP was found to be converted to DRP. This sample was collected in late summer of 1972, while all the other samples from the Genesee R. were collected in spring, so that possibly the nature of Sample No.16 was completely different from that of the spring samples.

The Black R. PP forms appeared to be quite consistent in terms of the percent of PP released as DRP after chloroform treatment. However, the values from the dark incubation systems (with and without resins) were low, except in the case of Sample No.25. This sample was collected in late summer of 1972. Again the difference could possibly be related to the time of collection, although the values from chloroform treatment were similar for all samples from the Black River.

Both the chloroform treatment and long-term dark incubations without resin were designed to measure the potentially available pool of phosphorus associated with microbial cells. The long-term dark incubations with resin were intended to measure, in addition, the inorganic P extractable from suspended soil particles. In nine of the 25 samples tested by dark incubation (with and without resin), the calculated DRP contribution from PP was zero, and the presence or absence of resin did not significantly affect the results for most of the 25 samples (Table 55). Only in seven of the 25 samples did the dark incubation values exceed the chloroform treatment values.

The algal-available P values measured in autoclaved, filtered samples (AAP test, EPA, 1971) appeared to be overestimates of the true available P, for reasons given in Section VI. After correction of the bioassay A_{750} values to a cell count basis to obtain corrected bioassay data, the corrected results (Table 58) were used in equation (1) to obtain the data shown in Table 61). In many samples, only slight changes in the TSP levels were noted as a result of autoclaving (Table 56), although such treatment should have produced roughly as much soluble P as the chloroform treatment because both methods resulted in microorganism cell lysis. In one sample, Niagara R. No.56, the TSP showed a marked decrease as a result of the autoclave treatment. Thus, the possibility exists that resorption of some of the P released by autoclaving of particulate

P forms in the sample could have caused some of the low values shown in Table 61.

Another possible reason for the low values from bioassays is the availibility of the TSP forms released during autoclaving. Table 62 shows the chemically measured DRP and TSP values compared to the bioassay values of some autoclaved, filtered river waters. In the case of the Niagara and Black R. samples, and Genesee Sample No.58, it appears that the bioassay values were closer to the DRP values than to the TSP values, indicating that perhaps some of the "soluble" P released from autoclaving was biologically not available to Selenastrum. Since these forms could only be measured after persulfate digestion, they may have been colloidal or soluble organic P esters, perhaps refractory cellular debris.

The net result of these factors was a relatively low calculated PP availability (equation 1) from bioassays of autoclaved, filtered river waters (AAP test) in comparison to direct bioassays of autoclaved suspensions of PP forms in AAP (-P) medium. This comparison is shown in Table 63. The combination of (a) resorption of soluble P released during autoclaving (and thus its loss via the filtration prior to bioassay) and (b) the apparently low availability of the soluble, unreactive P forms remaining after autoclaving was likely responsible for some of the low results found with the standard AAP test and equation (1). The direct algal bioassay of autoclaved PP forms did not involve a filtration prior to bioassay, so that any resorbed P was still potentially available to the test algae, as was the inorganic P originally associated with the PP forms. However, in both assay procedures, the considerations expressed in part (b), above, would apply, since in both

Table 62. COMPARISON BETWEEN DRP, TSP, AND ALGAL-AVAILABLE P IN AUTOCLAVED, FILTERED NEW YORK RIVER WATERS

Sample No.	DRP	TSP	Algal-Available P[a]
	(μgP/L)		
	Niagara R. at Ft. Niagara		
50	2	16	<4
56	6	17	7
	Niagara R. at Beaver I. Park		
49	1	16	<4
57	5	15	4
	Genesee R.		
51	103	113	124
58	52	68	57
	Oswego R.		
52	52	73	63-67
59	48	76	65
	Black R.		
53	18	37	23
60	30	51	38

[a] Corrected values for all samples except those of the Black R.; values from Table 58.

Table 63. PERCENT OF RIVER WATER PP AVAILABLE TO SELENASTRUM IN DIRECT BIOASSAYS OF AUTOCLAVED PP, AS COMPARED TO CALCULATED PP AVAILABILITY IN BIOASSAYS OF AUTOCLAVED, FILTERED RIVER WATER

	Percent of PP Available to Selenastrum	
Sample No.	Direct bioassay of autoclaved PP from river water[b]	Calculated[a] from bioassay of autoclaved, filtered sample of river water
		Niagara R. at Ft. Niagara
50	57	0
56	63	0
		Genesee R.
51	41	21
58	36	0
		Oswego R.
52	44	15-33
59	32	7
		Black R.
53	45	28
60	26	19

[a] Calculated from data in Table 58 and equation (1) of the text.

[b] Data from Table 49.

methods the soluble P forms remaining after autoclaving are the same. Thus, the differences seen in Table 63 between the two methods must have been the result of the filtration step used in the standard AAP test.

The foregoing comparison indicates that the standard AAP test (EPA, 1971) for estimating the potential available P from both soluble and particulate P forms in natural waters may underestimate the contribution of available P from the insoluble forms. The direct assay of particulate forms isolated from the parent sample and autoclaved in AAP(-P) medium probably is a better procedure for determining the potential available P from PP, especially since both the sample and standard cultures contain essentially the same growth medium, AAP(-P), whereas in the standard AAP test, the samples contain AAP(-P) medium plus the nutrient salts from the river water.

Total P Availability

The maximum observed values of DRP or algal-available P from the various treatments of river waters were also expressed as a percentage of the TP in the samples. The direct bioassays of autoclaved PP were fitted into this scheme by calculating the "A" values, as shown in equation (2):

$$A = \text{estimated available P in sample} \div \text{TP} \times 100\%$$

where:

$$\text{est. avail. P} = \text{sample DRP} + \left[\begin{array}{l}\text{\%PP avail.}\\ \text{to Selenastrum} \times\\ \text{sample PP after}\\ \text{autoclaving}\end{array}\right] \quad (2)$$

This equation assumes that the dissolved unreactive P (TSP - DRP) in the samples will not contribute significantly to the available fraction of TP. Since, in general, TSP - DRP was a relatively small part of the sample TP, the results calculated from equation (2) would not be greatly affected if this assumption were false. It can be stated that at least the P computed with equation (2) should be available.

Table 64 compares the availability computed from all the treatments of river waters, as well as the calculated "A" values from direct algal assays of autoclaved PP. Although the "S" value of TP availability from bioassay of autoclaved, filtered water had been shown (Table 65) to underestimate the PP availability of Samples Nos. 51, 52, and 59, the TP availability measured by the "S" and "A" values were in fairly close agreement for these samples. Since the PP in these samples was only a fraction of TP, differences in the measured PP availability would cause smaller relative differences in TP availability. In the Niagara R. samples, great differences in the "S" and "A" values were seen, because of the factors discussed above for the autoclaving-filtering ("S") procedure.

Figures 20 to 23 present the data from Table 64 in a graphic form. "S" values listed in Table 64 as "less than X" were plotted in the figures as a horizontal line at the value X, with an arrow labeled with an "S" pointing toward zero.

The Niagara R. samples (Figure 20) showed more variability in the maximum observed TP availability than did the other rivers. Chloroform treatment produced values of 26 to 91 percent of TP, while the dark incubations showed 12 to 51 percent of TP as DRP, and the AAP bioassays showed very low values of 15 percent or less. The "A" value for Sample No. 50 fell within the interval between the chloroform value and the dark incubation value, while the "A" value for Sample No. 56 was slightly higher than the

Table 64. COMPARISON OF CHEMICAL AND BIOLOGICAL TESTS FOR DETERMINING THE AVAILABILITY OF TP IN NEW YORK RIVER WATERS

	Maximum Mean DRP (% of TP)				Algal-Available P (% of TP)	
Sample No.	Initial DRP	After chloroform added[a]	After dark incubation[b]	After autoclaving	S Value[c]	A Value[d]
			Niagara R. at Ft. Niagara			
27	22	56	--	--	< 11	--
33	15	41	35	--	15	--
41	18	91	23	--	< 9	--
50	4	73	31	8	< 15	46
56	44	52	51	10	12	59
			Niagara R. at Beaver I. Park			
32	7	--	20	--	< 7	--
40	40	80	--	--	< 13	--
49	4	47	28	2	< 8	--
57	4	26	12	6	5	--
			Genesee R.			
16	26	--	70	--	--	--
34	7	9	16	--	8	--
42	27	34	28	--	21	--
51	60	71	64	60	72	75
58	24	38	37	26	28	50
			Oswego R.			
22(r.mouth)	62	79	--	--	--	--
23	51	69	79	--	--	--
24(r.mouth)	47	67	69	--	--	--
26	51	62	74	--	--	--

Table 64. COMPARISON OF CHEMICAL AND BIOLOGICAL TESTS FOR DETERMINING THE AVAILABILITY OF TP IN NEW YORK RIVER WATERS

	Maximum Mean DRP (% of TP)				Algal-Available P (% of TP)	
Sample No.	Initial DRP	After chloroform added[a]	After dark incubation[b]	After autoclaving	S Value[c]	A Value[d]
28	73	74	--	--	66	--
29	74	81	--	--	83	--
31	45	52	67	--	38	--
35	45	64	70	--	84	--
43	40	79	69	--	41	--
47	42	--	--	--	58	--
52	48	69	61	50	60-69	68
54	46	67	59	--	--	--
55	36	62	--	--	--	--
59	31	62	46	33	44	50
			Black R.			
25	26	45	55	--	--	--
36	21	44	29	--	35	--
44	26	50	21	--	--	--
53	12	51	20	44	56	39
60	13	36	24	30	38	33

[a] Data from Table 52.

[b] Value given is the larger of the two values found with and without anion exchange resin added to the samples; data from Table 55.

[c] S values are the results of Selenastrum bioassays of autoclaved, filtered river water; data from Table 58.

[d] A values are derived from equation (2) in the text, using PP bioassay (autoclaved PP) data from Table 49.

Figures 20 to 23. Comparison of chemical and biological tests for determining the availability of TP in New Yor[illegible] river waters

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KEY:

- Ⓘ INITIAL DRP IN THE SAMPLE (Mean Value)
- ▲ DRP AFTER CHLOROFORM ADDITION(Maximum Observed Mean Value)
- □ DRP AFTER DARK INCUBATION (Maximum Observed Mean Value)
- ● DRP AFTER AUTOCLAVING (Mean Value)
- Ⓢ S VALUE ; MEAN VALUE OF *SELENASTRUM* BIOASSAY OF AUTOCLAVED, FILTERED RIVER SAMPLE (see Table 64)[a]
- Ⓐ A VALUE; COMPUTED FROM BIOASSAY OF PP FORMS IN AAP(-P) SUSPENSIONS (see Table 64)

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[a] Values of S in Table 64 which are listed as <x are shown in the following figures by a line drawn at x and an arrow pointing toward zero.

Figure 20.

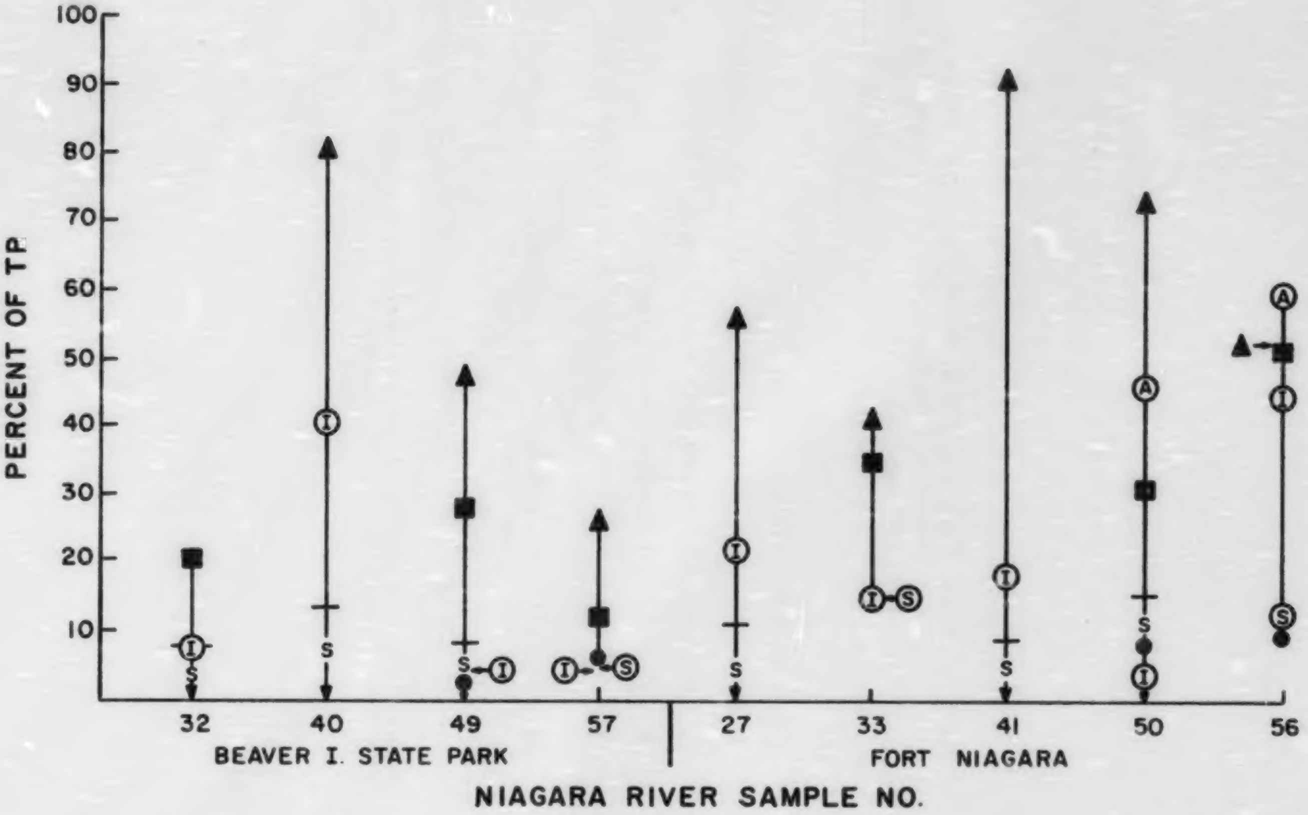

PERCENT OF TP
100
90
80
70
60
50
40
30
20
10
32
40
49
57
27
33
41
50
56
BEAVER I. STATE PARK
FORT NIAGARA
NIAGARA RIVER SAMPLE NO.
I
S
A

Figure 21.

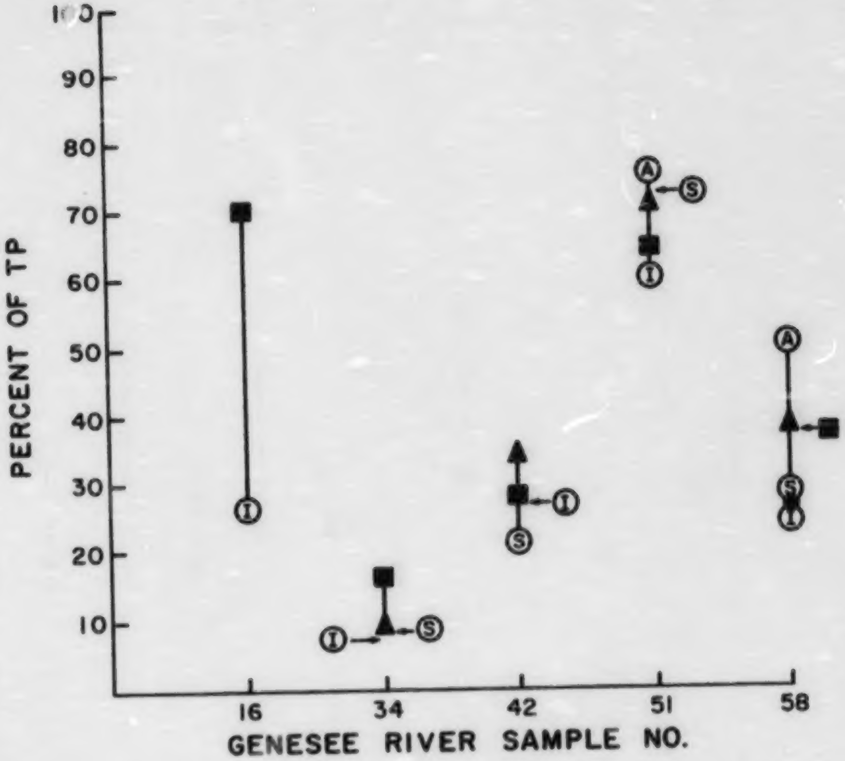

PERCENT OF TP
100
90
80
70
60
50
40
30
20
10
16
34
42
51
58
GENESEE RIVER SAMPLE NO.
I
S
A

Figure 22.

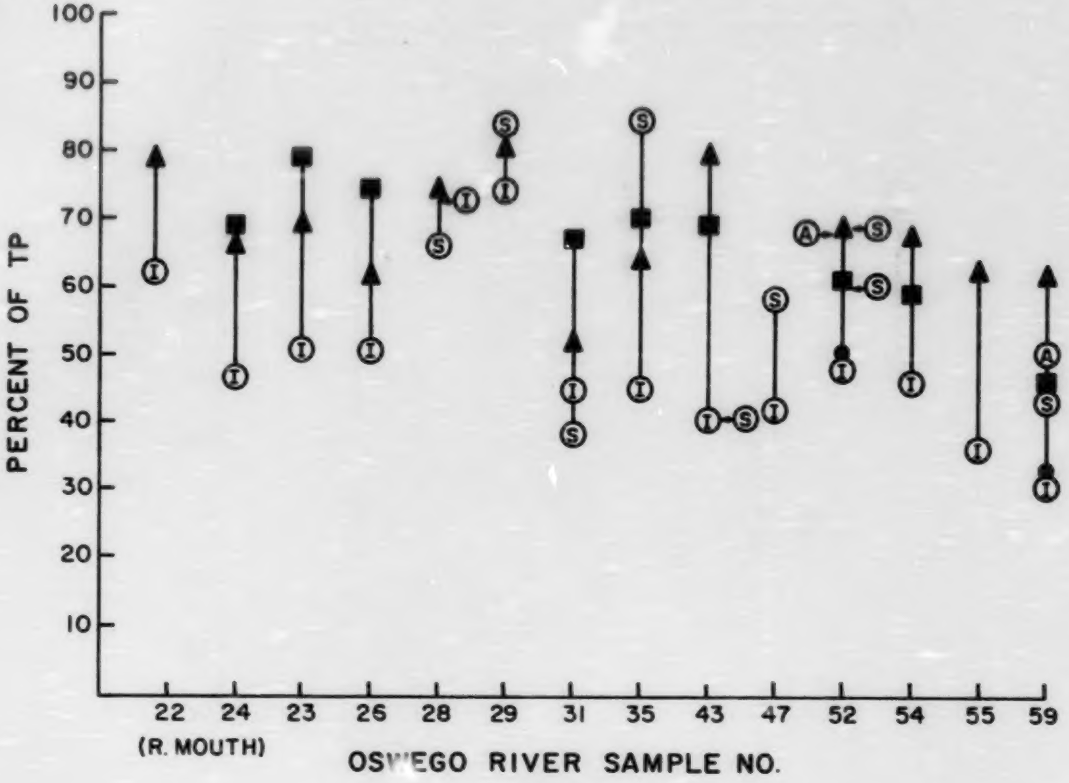
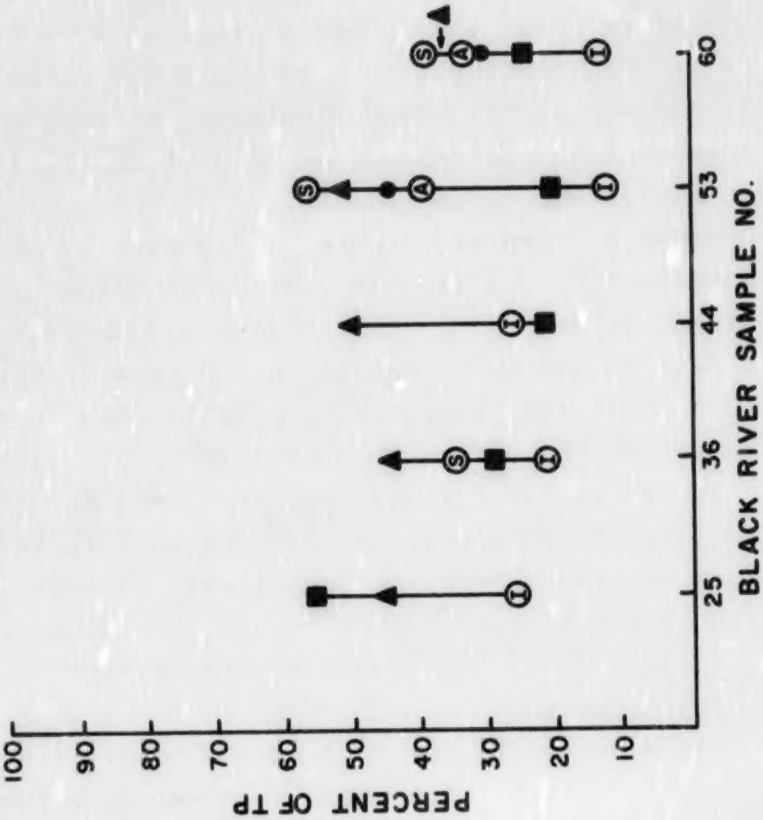

PERCENT OF TP
100
90
80
70
60
50
40
30
20
10
22
(R. MOUTH)
24
23
26
28
29
31
35
43
47
52
54
55
59
OSWEGO RIVER SAMPLE NO.
I
S
A

Figure 23.


PERCENT OF TP
100
90
80
70
60
50
40
30
20
10
BLACK RIVER SAMPLE NO.
25
36
44
53
60
S
A
I

chloroform value. The initial DRP values in these samples were less than 50 percent of TP; in all samples tested by chloroform or dark incubation treatments the initial DRP was exceeded by the DRP after treatment.

Like the Niagara R. samples, the Genesee R. samples (Figure 21) showed a wide range of observed TP availability because of the changing nature of the PP in the samples and the changing concentrations of soluble P forms in the samples. Chloroform treatment showed a range of 9 to 71 percent of TP as DRP, similar to the bioassay "S" value range of 8 to 72 percent of TP. The calculated "A" values were within 13 percent of the chloroform or dark incubation values, for Samples No. 51 and No. 58.

The Oswego R. samples (Figure 22) generally showed fairly consistent TP availability, with chloroform values between 52 and 81 percent of TP and dark incubation values between 46 and 79 percent. Except in the case of Samples No. 31 and No. 43, the "S" values generally were in the same range as the chloroform or dark incubation values. The range of "S" values for all samples except No. 31 and No. 43 was 58 to 83 percent. The "A" values fell into the interval between the chloroform values and the dark incubation values, for Samples No. 52 and No. 59. The two river mouth samples did not appear to differ significantly from the river samples.

Black R. samples (Figure 23) showed relatively consistent TP availability, with chloroform values between 36 and 51 percent of TP, and dark incubation values between 20 and 55 percent of TP. The "S" values agreed closely with the chloroform treatment values, but were higher than the dark incubation values. The "A" values fell between the chloroform and dark incubation values for Samples No. 53 and No. 60.

In summary, the Oswego and Black River samples tested for TP availability showed relatively consistent values between samples collected at various times during these studies. The Niagara and Genesee samples showed greater variability, such that an intensive sampling schedule would have to be established to adequately characterize the availability of P forms in these rivers throughout even the spring flow period. These studies indicate that generalizations about either PP or TP availability in the New York rivers would have to be weighed to account for the TP or PP loading to Lake Ontario for the time interval during which the measurements of availability were made. Only in this way could an accurate nutrient budget, based on [(concentration) X (% availability) X (flow)] data be constructed.

SECTION IX

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